"STUDY OF *IN VITRO* CYTOTOXICITY AND *IN VIVO* ANTI- TUMOUR AND ANTI-INFLAMMATORY ACTIVITIES OF *GMELINA ARBOREA* ROXB. STEM BARK"

Thesis Submitted to

The Tamilnadu Dr.M.G.R Medical University, Chennai

In partial fulfillment of the requirements

for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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APRIL – 2014

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled "**Study of *in vitro* cytotoxicity and *in vivo* anti- tumour and anti-inflammatory activities of *Gmelina arborea* Roxb. stem bark"** submitted by **Ms. Nify Faustine (Reg.No:261225656)** to **The Tamilnadu Dr. M.G.R. Medical University, Chennai** in partial fulfillment for the Degree of **Master Of Pharmacy** in **Pharmacology** is a bonafide work carried out during the academic year 2013-2014 by the candidate at the **Department of Pharmacology**, **R.V.S College of Pharmaceutical Sciences**, Sulur, Coimbatore and was evaluated by us.

Examination centre:

Date:

Internal Examiner **External Examiner**

CERTIFICATE

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DECLARATION

I **Nify Faustine,** hereby declare that the dissertation work entitled "**Study of *in vitro* cytotoxicity and *in vivo* anti- tumour and anti-inflammatory activities of *Gmelina arborea* Roxb. stem bark"** submitted by me, in partial fulfillment of the requirements for the degree of **Master of Pharmacy** in **Pharmacology** to **The Tamilnadu Dr.M.G.R Medical University, Chennai** is the result of my original and independent research work carried out under the guidance and supervision of **Mrs. C. Maheswari,** M.Pharm, (Ph.D) during the academic year 2013-2014 and this has not formed the basis for the award of any Degree/ Diploma/ Fellowship or similar title to any candidate of any university.

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(Nify Faustine)

DEDICATED TO MY FAMILY

LIST OF ABBREVIATIONS

%	:	Percentage sign
l	:	micro litre
^{0}C	:	Degree celsius
ANOVA	:	Analysis of variation
cm^3	:	Cubic centimetre
Fig	:	Figure
Tab	:	Table
gm	:	Gram
G.arborea	:	*Gmelina arborea*
hr	:	Hour
i.p	:	Intraperitonially
m mole	:	millimoles
mg/dl	:	milligrams per decilitre
mg/kg b.wt	:	milligram per kilogram body weight
mg/kg	:	1 milligram per kilogram
min	:	minute
sec	:	seconds
ml	:	Milli liter
mm	:	Millimeter
mm^3	:	Cubic millimetre
nm	:	nanometer

OD	:	Optical density
T.vol	:	Tumor volume
Hb	:	Hemoglobin
SD	:	Standard deviation
WBC	:	White blood cells
DLA	:	Dalton’s lymphoma ascites
EAC	:	Ehrlich ascites carcinoma
PBS	:	Phosphate buffer saline

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INTRODUCTION

Inflammation can be defined as "a generalized and nonspecific but beneficial response of tissues to injury". Inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality", or "the reaction to injury of the living microcirculation and related tissues (Spector *et al*., 1963). It comprises a complex array of adaptive responses to tissue injury which are both local and systemic.

The local responses may result in recruitment of phagocytic cells and removal of endogenous or foreign material. The systemic responses may alter the 'milieu interior' to allow these processes to occur more efficiently (Denko *et al*., 1992, Henson; Murphy., 1989).

The main pathophysiological pathways for drug targeting at present are: arachidonic acid metabolism; the complement cascade; phagocytosis and other cell functions; auto-immune processes; protein kinase C and others enzymes involved in second messenger systems (Willianson, 1996). Early inflammation changes in damaged tissues are now known to involve in the release of various biologically active materials from the polymorph nuclear leukocytes, the lysosomal enzymes and others. In general, the vascular effects may primarily mediated by kinins, prostaglandins and vaso-active amines (e.g. histamine, released by mast cells), which may cause an increased vascular permeability leading to plasma exudation.

The inflammatory process involves a complex interplay between the cells of blood, and the blood vessels themselves and also the cells of the tissue involved. The process can be seen as a coordination response of a large number of cells to an initial stimulus. The immigrating cells themselves exert little effect by their presence alone, but it can initiate the entire complex reaction of inflammation as a consequence of the materials that they secrete or release to the extra-cellular environment. Such materials include the molecules that exacerbate the responses by attracting the inflammatory cells, and the inhibitors that serve to reduce the severity of reactions, the histotoxic agents such as proteases, oxygen metabolites and cations, as well as signals the surrounding inflammatory and tissue cells to implement some or all of the complex reactions which they are capable (Henson *et al*., 1989).Uncontrolled inflammation is an

undesirable. The reversible features such as pain, redness, heat and swelling are joined by a fifth and less transient feature namely, loss of function of the organs involved. Therefore the control of inflammation is sought to protect the body function (Denko *et al*., 1992).

Causes

The factors that can stimulate an inflammation include microorganisms, physical agents, chemical substances, inappropriate immunological responses and tissue death. Infectious agents such as viruses and bacteria are some of the most common stimuli of inflammation. Viruses could give rise to inflammation by entering and destroying cells of the body; bacteria releases substance called endotoxins that can initiate inflammation. Inflammation can also occur by physical trauma such as burns, radiation, and frostbite which can damage tissues, and by corrosive chemicals such as acids, alkalis, and oxidizing agents. As mentioned above, malfunctioning of immunological responses can incite an inappropriate and damaging inflammatory response. Inflammation can also result when tissues die due to lack of oxygen and nutrients, a situation that could often is caused by loss of blood flow to the area.

Signs

The four cardinal signs of inflammation are redness, heat , swelling , and pain. These were mentioned in the 1st century AD by the Roman medical writer Aulus Cornelius Celsus. Redness is caused by dilation of the small blood vessels at the site of injury. Heat results from increased blood flow through the site and is experiences only in the peripheral parts of body such as skin. Fever is also brought by chemical mediators of inflammation by rising the temperature at the injury. Swelling which is called as edema is caused by the accumulation of fluid outside the blood vessels. The pain accompanied with inflammation results in part from the damage of tissues caused by edema, and it is induced by certain chemical mediators of inflammation like bradykinin, serotonin, and the prostaglandins.

A fifth effect of inflammation is loss of function of the inflamed area, a charecter noted by German pathologist Rudolf Virchow in the 19th century. Loss of function results from pain that inhibits movement or from severe swelling that prevents movement in the area.

The acute inflammatory response

Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A series of biochemical events is propagated and matures the inflammatory response, involving the the immune system, local vascular system and various cells within the injured tissue. Prolonged inflammation which is known as chronic inflammation, leads to a advancing shift in the type of cells present at the area of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

Acute inflammation is a short term process, normally appearing within a few minutes or hours and terminating upon the removal of the injurious stimulus. The process of acute inflammation is initiated by cells present in all the tissues mainly dendritic cells, Kupffer cells, resident macrophages, histocytes and mastocytes. These cells are present on their surfaces contain receptors named pattern recognition receptors (PRRs), and these recognize molecules that are shared by pathogens but distinguishable from host molecules, collectively called as pathogen-associated molecular patterns (PAMPs). At the beginning of an infection, injury or burn, these cells gets activated (one of their PRRs recognize a PAMP) and inflammatory mediators are released responsible for the clinical signs of inflammation. Vasodilation causes increased blood flow which cause the redness and increased heat. The increased permeability of the blood vessels results in exudation or leakage of plasma proteins and fluid into the tissue which manifests itself as swelling. Some of the mediators released such as bradykinin increase the sensitivity to pain which is reffered to hyperalgesia. The mediator molecules alters the blood vessels to permit the migration of neutrophils outside of the blood vessels into the tissue. The neutrophils migrate along a chemotactic gradient created by local cells to reach the site of injury. The loss of function (*functio laesa*) is probably the result of a neurological reflex in response to pain.

In addition to cell-derived mediators various acellular biochemical cascade systems consisting of plasma proteins acts in parallel to initiate and transmit the inflammatory response. These include the complement system , the coagulation and fibrinolysis systems activated by necrosis, e.g. a burn or a trauma. The acute inflammatory response requires constant stimulation to be

maintained. Inflammatory mediators have short half lives and are degraded quickly in the tissue. Therefore, acute inflammation stops once the stimulus has been removed.

Vascular changes

When tissue is first injured the small blood vessels in the damaged area constrict momentarily, a process called vasoconstriction. Following this temporary event, the blood vessels dilate (vasodilation), increasing blood flow into the site. Vasodilation may last from fifteen minutes to several hours.

Next the walls of the blood vessels become more permeable, which allow only water and salts to pass through easily. Protein-rich fluid called exudate, is able to exit into the tissues. Substances in the exudate consists of clotting factors and these factors help prevent the spread of infectious agents throughout the body. Other proteins are antibodies that help in destroying the invading microorganisms.

As the fluid and other substances leak out of the blood vessels, blood flow becomes more inactive and white blood cells begin to fall out of the axial stream in the centre of the vessel to flow near the vessel wall. The white blood cells then stick to the blood vessel wall which is the first step in their emigration into the extravascular space of the tissue.

Cellular changes

The chief feature of inflammation is the accumulation of white blood cells at the site of injury. Most of these cells are phagocytes, leukocytes that ingest bacteria and other foreign particles and also clean up cellular debris caused by injury. The main phagocytes engaged in acute inflammation are the neutrophils which are, a type of white blood cell that contains granules of enzymes destroying cells and proteins. When tissue damage is less, an adequate supply of these cells can be acquired from those already circulating in the blood. When damage is more, some immatured neutrophils are released from the bone marrow where they gets generated.

To perform the tasks, not only neutrophils must exit through the blood vessel wall but they must actively move from the blood vessel toward the area of tissue damage. This movement is made by chemical substances that diffuse from the site of tissue damage and create a concentration gradient followed by neutrophils. The substances which create the gradient are called Chemotactic factors and the migration of cells along the gradient is called Chemotaxis. Large numbers of neutrophils reach the site of injury first, sometimes within an hour after injury or

infection. After the neutrophils, often 24 to 28 hours after inflammation begins, there comes another group of white blood cells, the monocytes, which eventually mature into cell-eating macrophages. Macrophages usually become more prevalent at the site of injury only after days or weeks and are a cellular hallmark of chronic inflammation.

Chemical mediators of inflammation

Although injury initiates the inflammatory response, chemical factors released upon this stimulation bring about the vascular and cellular changes described above. The chemicals originate from white blood cells (basophils, neutrophils, monocytes, macrophages), blood plasma, platelets, mast cells, damaged tissue cells and endothelial cells lining the blood vessels. One of the best-known chemical mediators released from cells during inflammation is histamine which give rise to vasodilation and increases vascular permeability. Histamine is released immediately stored in granules of circulating basophils and mast cells, when these cells are injured. Other substances involving in the increased vascular permeability are lysosomal compounds released from neutrophils. The cytokines secreted by cells involved in inflammation also contribute vasoactive and chemotactic properties.

The prostaglandins are a group of fatty acids produced by many types of cells in which some of them increase the effects of other substances that promote vascular permeability. Other prostaglandins affect the aggregation of platelets which is the major part of the clotting process. Prostaglandins are the cause or they are associated with the pain and fever of inflammation. Anti-inflammatory drugs such as aspirin are effective in this because they inhibit the enzyme involved in prostaglandin synthesis. Prostaglandins are synthesized from arachidonic acid, as are the leukotrienes, another group of chemical mediators that have vasoactive properties.

The plasma contains four interrelated systems of the kinins, proteins—complement, coagulation factors and the fibrinolytic system, that generate various mediators of inflammation. The activated complement proteins serve as chemotactic factors for neutrophils,, stimulate the release of histamine from mast cells and increase vascular permeability They also adhere to the surface of bacteria. This makes them easier targets for phagocytes. The kinin system is activated by coagulation factor XII and produces substances that increase vascular permeability. The most important of the kinins is bradykinin, which causes much of the pain and itching experienced

with inflammation. The coagulation system converts the plasma protein fibrinogen into fibrin. This is a major component of the fluid exudate. The fibrinolytic system, contributes to inflammation primarily through the formation of plasmin which breaks down fibrin into products that affect vascular permeability.

Events following acute inflammation

Once acute inflammation has initiated, a number of events may follow. These include healing and repair, suppuration, and chronic inflammation. The events depends on the type of tissue involved and the amount of tissue destroyed, which are in turn related to the cause of the injury.

Healing and Repair

During the healing process the damaged cells which are capable of proliferation regenerate. Different types of cells varies from one another in their ability to regenerate. The cells such as epithelial cells will regenerate easily, while other cells like liver cells does not normally proliferate but can be stimulated to do so after damage has occurred. Various other types of cells are incapable of regeneration. Repair, which occurs when tissue damage is substantial, results in the formation of a fibrous scar. Through the repair process the endothelial cells gives rise to new blood vessels, and cells called fibroblasts that grow to form a loose framework of connective tissue. This delicate vascularized connective tissue is called granulation tissue. It derives its name from the small red granular areas that are seen in healing tissue (e.g., the skin beneath a scab).

Suppuration

The process of pus formation is called suppuration and it occurs when the agent that provoked the inflammation cannot be eliminated. Pus is a viscous liquid that consists of dead and dying neutrophils and bacteria, fluid leaked from blood vessels , and cellular debris. The most common cause of suppuration is infection with the pyogenic or pus-producing bacteria such as *Streptococcus* and *Staphylococcus*.

Once pus begins to collect in a tissue, it becomes surrounded by a membrane and gives rise to a structure called an abscess. Because an abscess is virtually not accessible to antibiotics and antibodies and its very difficult to treat and a surgical incision is needed to drain and eliminate it.

Some abscesses such as boils will burst of their own. The abscess cavity then breaks down and the tissue is replaced through the process of repair.

Chronic inflammation

If an agent causing inflammation cannot be removed or if there is some hinderance with the healing process, an acute inflammatory response may develop to the chronic stage. Repeated occurrence of acute inflammation also can give rise to chronic inflammation. The duration, physical extent and the effects of chronic inflammation vary with the cause of the injury and the body's ability to improve the damage.

Some of the most common and disabling human diseases like rheumatoid arthritis, tuberculosis and chronic lung disease are charecterized by this type of inflammation. Chronic inflammation can be caused by infectious organisms that are able to resist host defences and continues in tissues for an extended period. These organisms include protozoa, fungi, metazoal parasites and *Mycobacterium tuberculosis* which is the causative agent of tuberculosis. Other inflammatory causative agents are materials foreign to the body that cannot be removed by phagocytosis or enzymatic breakdown such as substances that can be inhaled like silica dust, metal that can enter into wounds or wood splinters. In autoimmune reactions the stimulus to chronic inflammation is a normal component of the body to which the immune system has become sensitized. Autoimmune reactions give rise to chronic inflammatory diseases such as rheumatoid arthritis.

The indication of chronic inflammation is the infiltration of the tissue site by plasma cells, lymphocytes, and macrophages (mature antibody-producing B lymphocytes). These cells are taken up from the circulation by the steady release of chemotactic factors. Macrophages are the chief cells involved in chronic inflammation and produce many effects that contribute to the progression of tissue damage and to consequent functional impairment. Granulomatous inflammation is a distinct type of chronic inflammation and is marked by the formation of granulomas which are small collections of modified macrophages called epithelioid cells and are surrounded by lymphocytes. Granulomas contain, cells that form from the coalescence of epithelioid cells which are giant or Langhans. A definitive example of granulomatous inflammation is tuberculosis, and the granulomas formed are called tubercles.

Granulomas also typically arise from fungal infections, and they are present in schistosomiasis, syphilis, and rheumatoid arthritis (Kara Rogers, 2009).

Types of Chronic inflammation: Unspecific (e,g : chronic peptic ulcer) and specific (granulomatous).According to the mechanism, granulomatous inflammation may be: immune type (tuberculosis, sarcoidosis) and non-immune type (foreign body reaction).

Classification of granulomatous inflammation based on the etiology:

1. Infectious granuloma:
    1. .Bacterial:
        a. Mycobacterium tuberculosis
        b. Mycobacterium leprae
        c. Treponema pallidum
        d. Gram-positive bacillus
        e. Gram-negative bacillus
    2. Parasitic
        a. Toxoplasma gondii- Toxoplasmosis
        b. Helminths- Cysticerosis
    3. Fungi

11. Foreign body granuloma

111. Unknown etiology granuloma :
    1. Sarcoidosis
    2. Crohn' s disease

CANCER

Cancer is a group of diseases which is characterized by uncontrolled growth and spread of abnormal cells. If the spread of abnormal cells is uncontrolled it may lead to death. Cancer is caused by both external factors like tobacco, chemicals, radiation, infectious organisms and internal factors such as inherited mutations, immune conditions, hormones, and other mutations that occur from metabolism. These causative factors may act either together or in sequence to

initiate or promote carcinogenesis. Most of the cancers require several steps for their development which occur over many years.

According to the evaluation from the International Agency for Research on Cancer (IARC) (Ferlay *et al*., 2008), there were 12.7 million recent cancer cases in 2008 worldwide of which 7 million occurred in economically developing countries and 5.6 million occurred in economically developed countries.

TUMOUR

A tumour is commonly used as a synonym for a neoplasm (Saunders., 2007). It is a solid or fluid filled cystic lesion that may or may not be formed by an abnormal growth of neoplastic cells that appears enlarged in size. But tumor is not synonymous with cancer, cancer is malignant while a tumor can be benign, pre-malignant or malignant, or it can represent a lesion without any cancerous potential. Cancer stem cells may play an important role in tumor growth. Scientists believes that cancer might have its own stem cells that results in the re growth of tumours.

According to Medilexicon's medical dictionary (Nordqvist *et al.*, 2012), a Tumour is:

1. *Any swelling or tumefaction.*

2. *One of the four signs of inflammation enunciated by Celsus.*

CAUSES OF TUMOR:

In common, tumours (Moscow *et al.,* 2011) occur when cells divide immoderately in the body. The cell division is strictly controlled and the new cells are formed to replace existing or old ones to perform new functions. Cells which are damaged or not needed would die for healthy replacements. The common causes of tumour can be :

– If there is disturbance in the balance between cell division and death, a tumour may form.

– If there are problems with the body's immune system it can also lead to tumours.

– Tobacco causes more deaths than any other environmental pollutants. Other causes are due to:

- Benzene, other chemicals and toxins
- Drinking too much alcohol

- Environmental toxins, such as certain poisonous mushrooms and a type of poison that can grow on peanut plants (aflatoxins)
- Excessive sunlight exposure, genetic problems, obesity, radiation and viruses.

Tumours which are known to be caused by viruses are:

- Cervical cancer caused by human papilloma virus
- Hepato cellular carcinoma caused by hepatitis B virus

Some tumours are more common in one gender and some are more common among children than elderly and vice versa. Other causes are related to family history , diet, and environment.

SYMPTOMS OF TUMOUR:

Symptoms depends on the type and location of tumour. For example lung tumours may cause shortness of breath, coughing or chest pain and tumours of the colon may cause diarrhoea, weight loss, blood in the stool, constipation or iron deficiency anaemia.

Some tumours may not cause any symptoms until the developed stage. In certain tumours like pancreatic cancer, symptoms do not start until the disease has reached the chronic stage. The common symptoms of most tumours are sensation of coldness, fatigue, fever, weight loss, night sweats and loss of appetite.

TYPES OF TUMOUR

Tumours are the groups of abnormal cells that forms solid masses or growths. Tumours grow and behave differently depending on their type; non-cancerous (benign) or cancerous (malignant). Precancerous conditions may have the capability to develop into a cancer. Tumour can be classified into:

- Benign Tumour
- Premalignant Tumour
- Malignant Tumour

Benign Tumour:

Benign tumours (Mazumdar *et al*., 2001) are non-cancerous and grow very slowly which do not spread into other tissues. They are not usually life-threatening. They are not harmful if left alone. But some benign tumours may cause problems. Most benign tumours are not harmful to human health. But some may press against blood vessels or nerves and cause pain and other adverse effects. Benign tumours of endocrine tissues results in the excessive production of some hormones. Examples of benign tumors include:

- **Adenomas** – are tumours that arise from glandular epithelial tissue. Examples include adrenocortical adenoma, hepatocellular adenoma, basal cell adenoma, pituitary adenoma, chromophobe adenoma, bile duct adenoma, follicular adenoma, and nipple adenoma. Even though adenomas are non cancerous, there is a chance of becoming cancerous, then they are called adenocarcinomas.

- **Fibroids** (fibromas) - benign tumors that grow on fibrous or connective tissue of any organ in the body. Uterine fibroids are common. It is of two types-hard fibroma, which is made up of many fibers and few cells; and soft fibroma which is made up of several loosely connected cells and less fibroid tissue. Usually soft fibroma is found in the armpits, groin, neck and eyelids.Other types of fibromas are angiofibroma, cystic fibroma, myxofibroma, nonossifying and ossifying fibroma, cemento-ossifying fibroma, pleomorphic fibroma, etc. Some fibromas may cause symptoms and require surgical removal. Rarely, fibroids that change and eventually become cancerous are called fibrosarcomas.

- **Hemangiomas** - are benign tumors which consists of many blood cells. Sometimes they may be seen on the surface of the skin and are called *strawberry marks*. The majority of them appears at birth and they gradually goes with time. Usually they do not require any treatment. If they affects the patient's sense organs, the doctor may recommend treatment with corticosteroids. If the patient is above 10 years of age, they are commonly removed using laser surgery.

- **Lipomas** -are soft-tissue tumors. Lipomas consist of adipose tissue (fat cells). Most of them are very small, painless, movable and often soft to the touch. They are more common among people above 40 years. Experts disagree on whether lipomas change and become cancerous (malignant). Examples are angiolipoleiomyoma, angio lipoma, chondroid lipoma, intradermal spindle-cell lipoma, neural fibrolipoma, pleomorphic lipomas and superficial subcutaneous lipoma (most common type which is found just below the skin's surface).

Premalignant Tumor:

A premalignant (Ambrosi *et al*., 2002) or precancerous tumor is one that is not yet malignant or cancerous, but is about to become so. Examples of premalignant growths include:

- **Metaplasia of the lung** - the growths occur in the bronchi. The bronchi which are grandular can change and become squamous cells. Its major cause is smoking.

- **Dysplasia of the cervix** - the normal cells lining the cervix of the uterus change. The growth can be premalignant and may result in cervical cancer. Cervical dysplasia is usually diagnosed with PAP smear. According to the National Institutes of Health, USA, about 5% of PAP smears detect the presence of cervical dysplasia. They are common in women age group 25 to 35. They may be removed with cryotherapy (freezing), or conization (the cone of tissue from the cervix is removed).

- **Actinic keratosis** - also known as senile keratosis or solar keratosis, which is a premalignant growth consisting of crusty, scaly and thick patches of skin. Fair people are more susceptible to these types of growths, especially people who are exposed to sunlight. They are potentially premalignant because a number of them progress to squamous cell carcinoma. There is about 20% risk that untreated lesions may eventually become cancerous. However continuous sun exposure increases the risk of malignancy.

- **Leukoplakia** - thick, white patches on the gums, bottom of the mouth, inside cheeks and on the tongue (less commonly). They cannot be scraped off easily. Experts believe tobacco smoking or chewing is the main cause. As leukoplakia is rarely dangerous, a small percentage is premalignant and can eventually become cancerous. Many mouth cancers occur in the areas next to leukoplakia.

Malignant Tumor:

Malignant tumors (Mazumdar *et al*., 2001) are cancerous tumors, they tends to become progressively worse, and can result in death. Unlike benign tumors, malignant grows fast, and they spread (metastasize).Metastasis is the process by which cancer cells spread from their primary site to distant locations in the human body. For instance, a patient may have started off with melanoma (skin cancer) which metastasized in their brain. There are many types of tumors, which are made up of specific types of cancer cells:

- **Carcinoma** - these tumors are derived from the skin or tissues that line body organs (epithelial cells). For example, carcinomas can be of the lung, liver, stomach, pancreas, colon, prostate or breast. Many of the most common tumors are of this type, especially among the older patients.

- **Sarcoma** - these are tumors that start off in connective tissue, such as bones,cartilage, fat and nerves. They originate from the mesenchymal cells outside the bone marrow. Majority of them are malignant. They are so called after the cell, tissue or structure they originates from, for instance osteosarcoma, angiosarcoma, liposarcoma, chondrosarcoma and fibrosarcoma.

- **Lymphoma/Leukemia** - cancer arises from the blood forming (hematopoietic) cells that originate in the marrow and generally mature in the blood or lymph nodes. Leukemia accounts for 30% of childhood cancers. Leukemia is the only cancer where tumors are not formed.

- **Germ cell tumor** - these are tumors that arise from germ cells or pluripotent cells. They are most commonly present in the ovary or testicle. The majority of testicular tumors are of germ cells. Less commonly, germ cell tumors may also appear in the brain, abdomen and chest.

- **Blastoma** - tumors derived from embryonic tissue or immature precursor cells. They are more common in children than adults. For example, medulloblastoma and glioblastoma are kinds of brain tumors, retinoblastoma is a tumor within the retina of the eye, an osteoblastoma is a type of bone tumor, while a neuroblastoma is a tumor mostly found in children of neural origin.

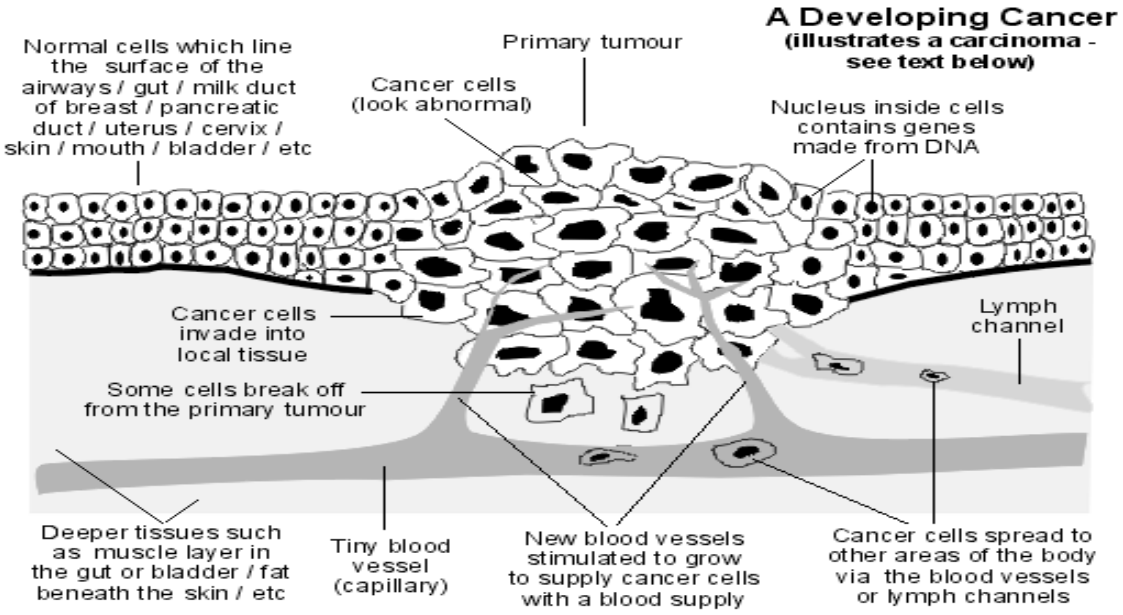


Fig.1: Stages of development of cancer

EXAMINATIONS AND TESTS FOR TUMOUR:

When a tumour is found, a biopsy is performed to determine if the tumour is noncancerous (benign) or cancerous (malignant). Depending on the location of the tumour, the biopsy can be a simple procedure or a serious operation. Most patients with tumours have CT or MRI scans to determine the exact location of the tumour and how far it has spread. Recently, positron emission tomography (PET) scans have been used to find certain tumour types.

Other tests include:

- Biopsy of the tumour
- Blood or Urine tests
- Bone marrow biopsy
- Endoscopy
- Chest X-ray
- Complete blood count (CBC)

TREATMENT OF TUMOR:

Treatment varies based on:

- The type of tumor
- Whether it is noncancerous or cancerous
- Its location

If the tumour is benign (meaning it has no potential to spread) and is located in a safe area where it will not cause symptoms or affect the function of the organ, sometimes no treatment is needed. Benign tumours of the brain may be removed because of their location or harmful effect on the surrounding normal brain tissue.

If a tumour is cancerous, possible treatments include:

- Chemotherapy
- Surgery
- Radiation
- A combination of these methods
- Complementary and alternative medicine

If the cancer stick to one location, the goal of treatment is usually to remove the tumour with surgery. If the tumour has spread only to local lymph nodes, sometimes these can also be removed. If the cancer cannot be removed with surgery, the options for treatment include radiation and chemotherapy or both. Some patients need a combination of radiation, surgery, and chemotherapy. Lymphoma is rarely treated with surgery. Radiation therapy and chemotherapy are most often used for treating lymphoma.

CHEMOTHERAPY

Chemotherapy (Tripathi., 2008) is an important modality in cancer treatment. Chemotherapy involves administering powerful chemical agents (drugs) to destroy cancer cells in the entire organism. It causes a greater proportion of cell death among neoplastic as opposed to normal cells. However damage to normal cells result in chemotherapy toxicities and side effects, it can be seen that those actively dividing cells are most vulnerable.

Antineoplastics are general category of drugs used in chemotherapy and they are classified as:

1. **Alkylating Agents** :
   - Nitrogen mustards -
     Cyclophosphamide (Cytoxam)
     Chlorambucil (Leukeran)
     Melphalan (Alkeran)
     Mechlorethamine hydrochloride (Mustargen)
     Ifosfamide (Ifex)
   - Alkyl sulphonates -
     Busulphan (Myleran)
   - Nitrosoureas -
     Carmustine (BCNU)
     Lomustine (CCNU)
   - Ethylenimines -
     Thiotepa
   - Triazenes -
     Dacarbazine (DTIC-Dome)
2. **Antimetabolites**
   - Folate antagonist -
     Methotrexate (Folex, Mexate)
   - Purine analogues -
     Mercaptopurine (Purinethol)
     Pentostatin (Nipent)
   - Pyrimidine analogues -
     Cytarabine (Cytosar-U)
     Fluorouracil
3. **Anthracycline Antibiotics**
   Doxorubicin, Daunorubicin
   Mithramycin, Actinomycin D
4. **Vinca Alkaloids**
   Vinblastin (Velban)
   Vincristine (Oncovin)
5. **Epipodophyllotaxins**
   Etoposide, Teniposide
6. **Taxanes**

Paclitaxel, Docetaxel

7. **Camptothecins**
   Irinotecan, Topotecan
8. **Enzymes**
   L-Asparaginase
9. **Hormone derivatives**
   Tamoxifen (Nolvadex), Prednisolone
   Ethinyl estradiol

CYCLOPHOSPHAMIDE

Cyclophosphamide is a nitrogen mustard alkylating agent from oxazophorines group (McEvoy., 2007) and it is one of the most popular anti cancer drug. It is a medicine used to treat severe inflammatory illnesses such as complicated systemic lupus erythematosus (SLE/lupus), polymyositis (muscle inflammation), scleroderma and vasculitis (inflamed blood vessels) like Wegener's granulomatosis. It is inactive as such; produces few acute effects, Transformation into active metabolites (aldophosphamide, phosphoramide mustard) occurs in the liver and a wide range of anti tumor actions is exerted.

Brand name : Cycloblastin, Endoxan, Cytoxan, cytophosphane

Molecular Formula : $C_7H_{15}Cl_2N_2O_2P$

Molecular Mass : 261.1

Mechanism of Action :

Cyclophosphamaide produce highly reactive carbonium ion intermediates which transfer alkyl groups to cellular macromolecules by forming covalent bonds. Alkylation results in cross linking or abnormal base pairing of DNA strand. Cross linking of nucleic acids with proteins can also take place.

Side effects:

Nausea and vomiting, alopecia, Carcinogenicity, mouth ulcers and skin rash, bone marrow depression, bladder inflammation, Infections, neurotoxicity and reduce fertility, Easy bruising/bleeding, joint pain, mouth sores, syndrome of inappropriate antidiuretic hormone (SIADH), unusual decrease in the amount of urine and unusual tiredness or weakness.

Dosage :

Cyclophosphamide (Novack *et al.*, 1971) can be taken by mouth as tablets or it can be given via a vein as an infusion or injection. For long-term treatment it is normally taken in tablet form. For adults and children, usual dose is 1 to 5mg/Kg body wt/day.

Uses :

Cyclophosphamide is used in the treatment of Hodgkin & Non-Hodgkin lymphoma, Multiple myeloma, Leukaemia, cutaneous T-cell lymphoma, neuroblastoma, retinoblastoma, cancer of the ovary and breast, small cell cancer of the lung and sarcoma and also immune diseases.

PREVENTION OF TUMOUR:

The risk of cancerous (malignant) tumours can be reduced by:

- Eating a healthy diet
- Exercising regularly
- Limiting alcohol
- Maintaining a healthy weight
- Minimizing the exposure to radiation and toxic chemicals
- Not smoking or chewing tobacco
- Reducing sun exposure, especially if you burn easily

TUMOUR MARKERS :

These are glycoproteins in the blood that can be detected by monoclonal antibodies. Highly elevated levels of a tumour marker (Sturgeon *et al*., 2009) will provide helpful information but improper use can have economic significance, cause patients additional anxiety and strain, and unessential investigations may be associated with side-effects and may delay correct diagnosis and treatment.

Uses

Each tumour marker has a variable profile of uses (Perkins *et al*., 2003)

- **Screening -** Screening tests require high sensitivity to detect early stage disease.
- **Disease staging** - For diagnosis and prognosis.

- **Monitoring for cancer recurrence** - When monitoring these patients, tumour marker levels should be determined only when there is a potential for meaningful treatment.
- **Assessing response to therapy**
  - Tumour marker values returning to normal may indicate cure contempt radiographic evidence of continous disease. In this circumstance, the residual tumour is often non-viable.
  - However an increase in tumour marker levels, associated with lack of clinical improvement, may indicate treatment failure.
  - Residual elevation after specific treatment usually indicates persistent disease.

Tab.1: Clinically important Tumor markers

SL.NO:	TUMOR MARKER	ASSOCIATED PRIMARY TUMOR
1	CA 27.29	Breast cancer
2	CEA	Colorectal cancer
3	CA 19-9	Pancreatic and biliary tract cancers
4	AFP	Hepatocellular carcinoma, Nonseminomatous germ cell tumors
5	b-hCG	Non-seminomatous germ cell tumors and Gestational trophoblastic disease
6	CA-125	Ovarian cancer

ROLE OF MEDICINAL PLANTS IN CHEMOTHERAPY

India is the largest producer of medicinal plants and is called the Botanical garden of the World. Medical information in the old Indian literatures includes several medicinal plants which are used for thousands of years under the indigenous system of medicine. About 45,000 plant species have been identified in India, out of which about 15,000 to 20,000 plants are having good medicinal value. However, among these plants, traditional communities use only about 7000-7500 plants for medicinal purposes. The Ayurvedic system of medicine uses about 700, Siddha

600, Unani 700 and the modern medicine uses about 30 medicinal plants for treating a variety of diseases in both man and animal. Only few medicinal plants are attracted by the scientists for investigating them as a remedy for tumour. More than 50% of all modern drugs in clinical use are of natural products. Many of them have been recognized to have the capability to include apoptosis in various tumour cells. According to the World Health Organization (WHO) estimates, more than 80% of the people in developing countries depend on traditional medicine for their primary health needs. Some medicinal plants and their vegetables, fruits and crops play an important role in cancer prevention. Consumption of huge amount of fruits and vegetables can prevent the development of cancer. Doctors recommends that people who likes to reduce their risk of cancer should eat several pieces of fruits and vegetables daily. Several plant-derived products exhibit potent antitumor activity against many cancer cell lines.

A good number of medicinal plants (Khare., 2007) are found mentioned in the ancient classical Ayurvedic texts 'Charaka Samhita', 'Astanga Hridaya Samhita' and 'Susruta Samhita'. But many of them are still to be properly identified.

Anticancer properties of plant derived or natural products:

Plants have a long history of use in the treatment of cancer. Hartwell, in his review of plants used against cancer (Cragg *et al*., 2005) lists more than 3000 plant species that have reportedly been used in the treatment of cancer. It is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources including plants.

Plant derived compounds have played an important role in the development of several clinically useful anticancer agents. Many of the anticancer agents including taxol, vinblastine, vincristine and topotecan are in clinical use all over the world. Vinblastine and vincristine from the Madagascar periwinkle, *Catharanthus roseus* (Apocynaceae), introduced a new era in using plant material as a medication for treatment. They were the first agents in clinical use for the treatment of cancer. Vincristine and Vinblastine are used in combination with other cancer drugs,as the treatment for various kinds of cancers, including breast and lung cancers, leukemias, lymphomas, advanced testicular cancer. A number of hopeful agents such as combrestatin, betulinic acid and silvesterol are in clinical or preclinical development.

Medicinal plants (Kaur *et al*., 2011) maintain the health and vitality of individual and also cure various diseases including cancer without toxicity. Natural products which are discovered from medicinal plants have played an important role in treatment of cancer. These plants possess good antioxidant and immunomodulatry properties leading to anticancer activity.

Since chemotherapy and radiation cause severe toxicity, herbal plants are becoming popular throughout the world nowadays, and are also used as a therapy for tumors or cancer. The antitumour (antineoplastic) activities of several medicinal plants (Sharma *et al*., 2009) have been reported by various authors. Many of these plants include:

- *Abrus precatorious*
- *Aglaia roxburghiana*
- *Cassia fistula*
- *Catharanthus roseus*
- *Crocus sativus*
- *Ervatamia heyneana*
- *Hygrophila spinosa*
- *Hippocratea murcantha*
- *Indigofera mysorensis*
- *Ocimum sanctum*
- *Olea polygama*
- *Plumbago rosea*
- *Podophyllum hexandrum*
- *Semecarpus anacardium*
- *Solanum dulcamara*
- *Terminalia arjuna*
- *Trigonella foenumgraecum*
- *Vanda parviflora*
- *Wedelia calendulacea*
- *Withania somnifera*
- *Zingiber capitatum* .

In the present study *Gmelina arborea* stem bark is used. It is a medicinal plant, belonging to the family Verbeneceae. The present study is aimed at the anti inflammatory and anti tumour potential of the plant *Gmelina arborea*. Experimental evidence indicates its anti inflammatory and anti tumour potential. Since inflammation is involved in cancer this may have better possibility to act as anti tumour agent. The present study is under taken to evaluate its effect on inflammation inducing agents and tumour cell lines in mice.

REVIEW OF LITERATURE

Many of the herbal medicines and their active ingredients have been identified as the potential modifiers of cancer (Sawadogo *et al* 2012). Herbal medicines are yielding important breakthroughs in the treatment and prevention of cancer and have been used first line in numerous cultures across the world (Kraft., 2009). Research indicates the various possible mechanisms of action of herbal medicines, and their biological components, which act alone to reduce cancer risk through their anti-oxidant (Shahin., 2008), anti-carcinogenic properties and their direct suppressive effect on carcinogen bioactivities. Medicinal plants have been used for healing and preventative health for thousands of years all around the world. Herbal medicines may be used prophylactically, symptomatically and accurately.

Treatment with herbal medicine is considered as the second method to fight cancer utilized by cancer patient in developed countries (Kennedy., 2005 and Ezeome., 2007). It is considered as first line in the developing countries because of their availability and the affordable cost. Many plants were well known for anticancer property such as Nigella sativa (black seed), Cinnamomum cassia (Cinnamon), Panax ginseng (Ginseng), Camellia sinensis (Green tea),

Allium sativum (garlic), Zingiber officinale (Ginger). Several experiments and case report studies have been done and most of the conducted studies also showed supportive results. Moreover, various clinical trials have been conducted for several herbal products (Gonzalez *et al.,* 2010).

Most of Cancer diseases (90%) are due to external factors, only 10% is due to genetic factors (Peto., 2001). Preventive medicine will be very effective in fighting cancer and reducing the prognoses. The herbal products are most suitable since they have traditional and experimental evidences from other alternative medicines. The herbal medicines only requires studies to be conducted to use as preventive medicines. Modern technolology can help us to conduct these studies because there are animals which can carry human genes and long epidemiological studies can be carried out.

Anti-Cancer Agents Derived from Plants in Clinical Use

3.1. Vinca Alkaloids

The first agents introduced in clinical use were vinca alkaloids such as vinblastine and vincristine, isolated from the *Catharanthus roseus* (Apocynaceae). These drugs were found during an investigation for the oral hypoglycemic agents. The plant was endemic to Madagascar, and samples used in the discovery of vincristine and vinblastin were collected in Philippines and Jamaica. Recently semi-synthetic analogues of vinca alkaloids are vinorelbine and vindesine. These are used alone or in combination with other chemotherapeutic agents to fight a variety of cancers. Vinblastine is used for the treatment of breast cancer, testicular cancer, lymphomas, leukemias, lung cancers and Kaposi's sarcoma. Vincristine had also showed efficacy against leukemia and acute lymphocytic leukemia. Of over 2069 anti-cancer clinical trials recorded by the National Cancer Institute, over 160 are the combinations of drugs with these agents against a range of cancers.

3.2. Podophyllotoxin Derivatives

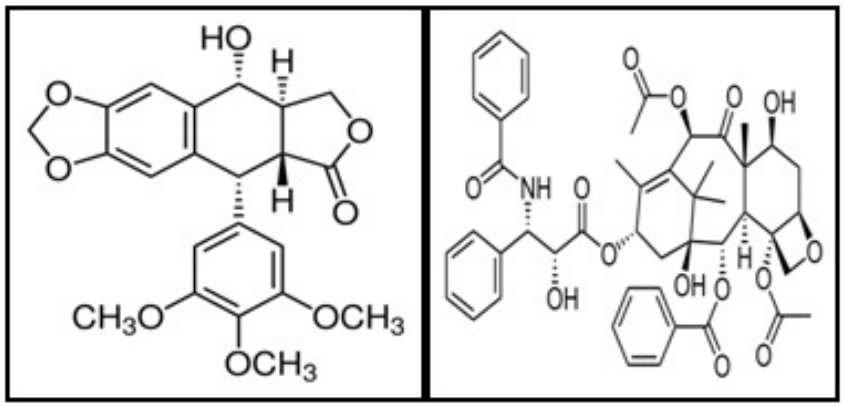
The species of Podophyllaceae family such as *Podophyllum peltatum* Linn., *P. emodii* Wallich have been reported with a history of therapeutical use. Extensive research studies in the 1960s and 1970s led to the development of etoposide and teniposide as clinical agents which are being used in the treatment of bronchial and testicular cancers. Of 2069 anti-cancer clinical trials recorded by the National Cancer Institute, over 150 are drug combinations with etoposide against a range of cancers.

3.3. Taxanes

A recent advancement in the development of plant derived chemotherapeutic agents is the development of a class called Taxanes. Paclitaxel also named as taxol was first isolated from the bark of *Taxus brevifolia* Nutt. (Taxaceae). Paclitaxel is used in the treatment of variety of cancers including ovarian, breast cancer and non-small-cell lung cancer, and also shown efficacy against Kaposi sarcoma. Docetaxel is a semisynthetic derivative which is used in the treatment of breast cancer. Of 2069 cancer clinical trials recorded by the National Cancer Institute, 248 are listed as involving taxane-derived drugs, including 134 with paclitaxel (Taxol), 105 with docetaxel (Taxotere), and 10 with miscellaneous taxanes, either as single agents or in combination with other anti-cancer drugs.

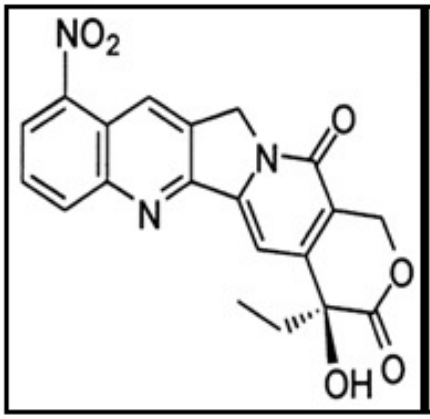
3.4. Campothecin Derivatives

Another advancement that was made in the anti-cancer drug is the class of clinically-active agents derived from Camptothecin. Campothecin was isolated from the Chinese ornamental tree, *Camptotheca acuminate* (Nyssaceae), and known as the tree of joy in china. The extract of *C. acuminata* was the only one out of 1000 various plant extracts tested for anti-cancer activity which showed safety and efficacy, and the active constituents was identified as Camptothecin. The derivatives are Topotecan and Irinotecan. Topotecan is used for the treatment of ovarian and small-cell lung cancers and Irinotecan is used for colorectal cancer treatment. Of the 2069 cancer clinical trials recorded by the National Cancer Institute, 94 are listed as involving camptothecin-derived drugs, including 64 with irinotecan (CPT-11), 26 with topotecan, and 4 with other miscellaneous analogues as single agents or in combination with other anti-cancer drugs and 15 other camptothecin derivatives are in preclinical development.

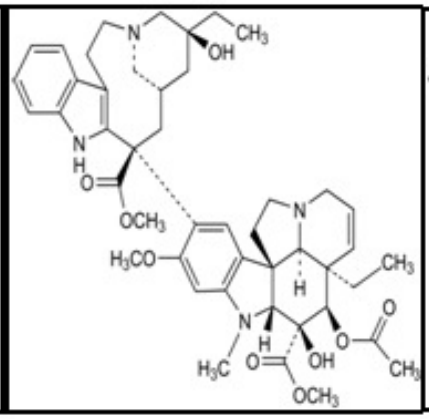


Podophyllotoxin

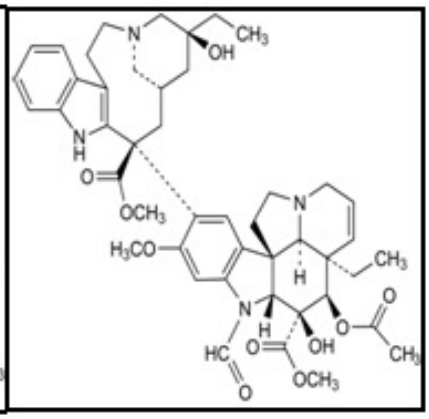
Taxol



Campothecin



Vinblastin



Vincristin

3.5. Homoharringtonine

Other plant-dericed agents which are in clinical use are Homoharringtonine. Homoharringtonine was isolated from the Chinese tree *Cephalotaxus harringtonia (Cephalotaxaceae).* Elliptinium was first isolated from species of the Apocynaceae family including *Bleekeria vitensis*, a Fijian medicinal plant with anti-cancer properties. In China, a racemic mixture of harringtonine and homoharringtonine (HHT) is being used successfully in acute and chronic myelogenous leukemia. Purified homoharringtonine is effective against certain leukemias and has been reported to produce complete hematologic abrogation in patients with late chronic phase chronic

myelogenous leukemia. Elliptinium is used in France for the treatment of breast cancer and is marketed (Om Prakash *et al*., 2013).

Tab.2 : Plant-derived anticancer agents

Sl.no:	Compound	Source	Mechanism	Cancer Use
1	Vincristine	*Catharanthus roseus*	mitotic block	Lymphoma, Leukemia, breast, lung cancers
2	Vinblastine	*Catharanthus roseus*	mitotic block	Breast, lymphoma
3	Paclitaxel	*Taxus brevifolia* Nutt, *Taxus baccata*	Anti-mitotic	Ovary, lung, breast, bladder
4	Docetaxel	*Taxus brevifolia* Nutt, *Taxus baccata*	Anti-mitotic	Breast and lung cancer
5	Topotecan	*Camptotheca acuminate*	DNA topoisomerase I inhibition	Ovarian, lung and pediatric cancer
6	Irinotecan	*Camptotheca acuminate*	DNA topoisomerase I inhibition	Colorectal and lung cancer
7	Flavopiridol	*Amoora rohituka* & *Dysoxylum binectariferum*	Inhibits cell cycle progression at G1 or G2 phase	colorectal, lung and renal cell carcinoma, non-Hodgkin's lymphoma, leukemia, solid tumors
8	Ellipticine	*Ochrosia borbonica*, *Excavatia coccinea*, *Ochrosia elliptica*	DNA intercalation and inhibition of topoisomerase II	Various cancer cell types
9	Etoposide and Teniposide	*Podophyllum peltatum* and *Podophyllum emodi*	not known	Lymphomas, bronchial and testicular cancers

GENERAL ADVERSE EFFECTS OF EXISTING ANTI CANCER DRUGS

Since most anticancer drugs act on the rapidly multiplying cells, they are also toxic to the normal multiplying cells in the bone marrow, epithelial cells of skin and mucous membranes lymphoid organs and gonads. Thus the common adverse effects are:

- Bone marrow depression resulting in leucopenia, thrombocytopenia and aplastic anaemia.
- Stomatitis, oesophagitis, glossitis and proctitis.
- Alopecia
- Depression of the immune system resulting in fatal infections such as typhilis.
- Infertility
- Teratogenicity
- Hyperuricemia- Rapid tumour cell lysis can result in an increased plasma uric acid levels and may precipitate gout.
- Carcinogenicity- Cytotoxic drugs themselves may cause secondary cancers
- Neurological adverse effects
- Immediate adverse effects like nausea and vomiting.
- Fatigue
- Cause bleeding by killing the rapidly dividing blood cells that reduce the number of platelets in the blood.

STUDIES OF PHYTOCHEMICALS RELATED TO CANCER DRUG DEVELOPMENT

The first agents to advance into clinical use were the vinca alkaloids, vinblastine (VLB) and vincristine (VCR) isolated from the *Catharanthus roseus* (Apocynaceae). This was used by various cultures of diabetes . While under the investigation as a source of potential oral hypoglycemic agents it was noted that extract reduced white blood cell counts and it caused bone marrow depression in rats and was found to be active against lymphocytic Leukemia in mice. This led to the isolation of VLB and VCR as active agents.

Podophyllum species (Podophyllaceae), *Podophyllum pelltatum* Linnaeus and *Podophyllum emodii* Wallich from the Indian sub continent had medical use including the treatment of skin cancers and warts (Lee *et al*., 2005).

Another important addition to the anti cancer armamentarium is the class of clinically active agents derived from Camptothecin which is isolated from the Chinese ornamental tree

Camptotheca acuminate. The two derivatives obtained from this are Topotecan and Irinotecan. Topotecan is used for the treatment of ovarian and small cell lung cancers while Irinotecan is used for the treatment of colorectal cancern (Rahier *et al .,* 2005*).*

In yet another antitumor study, foccused on flavanoids, polyphenolic compounds the role of dietary flavanoids in cancer prevention were widely discussed. Comparing data from laboratory studies, human clinical trials and epidemiological investigations, indicated that flavanoids have important effects on cancer chemoprevention and chemotherapy. Many different mechanisms of action was identified, including carcinogen inactivation, cell cycle arrest, anti proliferation, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multi drug resistance or a combination of these mechanisms (Ren *et al*., 2003).

Plant derived anti inflammatory agents :

Many hundreds of plants contain well known for their anti-inflammatory activities. Many herbs also shows anti-inflammatory (also known as antiphlogistic) characteristics. In toxicity studies, the anti-inflammatory plants shows great safety. Hundreds of plants contain well known anti-inflammatory effects. For example, Hops, an herb used in beer brewing, consists of a group of compounds called the humulones, which are being studied for their effective pain-relieving properties. It is sad when drugs that are commonly used are shown to pose great dangers to health. This is contradictory to their purpose. But the plant-based medicines is significantly advanced and now we have products available to us which will reduce or eliminate pain without being harmful to health. This is a good thing. As a result of the inherent problems associated with the current anti-inflammatory agents there is continous search from natural sources for possible agents. A wide variety of plants are employed in the treatment of inflammatory disorders by natural healers.

Some of these plants include

- *Aloe vera*
- *Consolida* regali
- *Chasmanthera dependens*

- *Culcasia scandens*
- *Crataeva religiosa*
- *Tanacetum vulgare*
- *Holmskioldia sanguine*
- *Mitracarpus scaber*
- *Turner ulmifolia*
- *Zingiber officinale*
- *Curcuma longa*
- *Azhadirachta indica*
- *Withania somnifera*

Most of these plants have shown varying activities in the various *in vivo* and *in vitro* inflammatory models. The potency of these plants belongs to several active principles present in them, which may act at any of the targets in the inflammatory response pathway. Besides anti-inflammatory activity, some of these plants also hold beneficial properties such as analgesic, antipyretic and antiulcer and antimicrobial effects,. These effects enhance the inherent anti-inflammatory activity and may grant advantage on these plants. Some active anti-inflammatory constituents of these plants have been identified and isolated. They include – lupeol, premnazole, (+) – usnic acid,(+) –pinitol, zanhasaponins A and B, sasanquol, parthenolide etc. These compounds could provide drugs with comparative advantage over existing agents..In addition, these medicinal plants will continue to serve as reservoir for development of potent drugs with less serious and life-threatening adverse effects (Okoli *et al*., 2002).

OBJECTIVES

- Preparation of methanolic extract and its fractions of *Gmelina arborea* stem and bark.
- Invitro cytotoxicity assay of *Gmelina arborea* extract using Trypan blue exclusion method.
- Analysis of anti inflammatory activity of *Gmelina arborea* extract using dextran induced and formalin induced acute and chronic inflammations.
- Analysis of *In vivo* antitumor activity of *Gmelina arborea* extract in mice using DLA induced solid tumor and EAC induced ascites tumuor model.

Plan of work

The plan of work for the study of *Gmelina arborea* was carried out as follows:-

Pharmacognosy studies:

- Collection of plant parts
- Authentication of plant materials.
- Successively solvent extraction by using Soxhlet apparatus.
- Preliminary phyto chemical screening.

Pharmacological studies:

- *In vitro* cytotoxic assay using DLA, EAC cell lines by Trypan blue exclusion method

- *In vivo* anti inflammatory activity of plant extract by
  a) Dextran induced acute inflammation
  b) Formalin induced chronic inflammation

- *In vivo* anti tumour activity of plant extract by
  a) DLA induced solid tumor model in mice
  b) EAC induced ascites tumour model in mice

.

1. PLANT PROFILE

Fig 2: Photograph of *Gmelina arborea* plant

TAXONOMICAL CLASSIFICATION

Table: 3 – Taxonomical classification of *G.arborea*

Kingdom	Plantae
Division	Magnoliophyta

Class	Magnoliopsida
Sub class	Asteridae
Order	Lamiales
Family	Verbenaceae
Genus	*Gmelina*
Species	*G. arborea*

VERNACULAR NAMES

Table : 4 – Vernicular names of *G.arborea*

Assamese	Gomari
Bengali	Gamari, Gambar
Hindi	Gamhar, Khamara.
Kannada	Kooli mara,Shivane mara, Kumbuda
Malayalam	Kumbil, Kumbulu
Marathi	Shivan, Siwan
Sanskrit	Gambhari, Sindhuparni
Tamil	Kumla, Kumalamaram
Telugu	Gumartek, Gummadi, Summadi

BOTANICAL DESCRIPTION

Gmelina arborea is a fast growing tree, which grows on different localities and prefers moist fertile valleys with 750–4500 mm rainfall. It does not thrive on ill-drained soils and remains stunted on dry, sandy or poor soils; drought also reduces it to a shrubby form.

The *Gmelina arborea* tree attains moderate to large height up to 30 m and its wood is pale yellow to cream coloured or plukish-buff when fresh, turning yellowish brown on exposure and is soft to moderately hard, light to moderately heavy, lustrous when fresh, usually straight to irregular or rarely wavy grained and medium course textured.

Leaves are large, tomentose underneath, cordate-ovate, acuminate.

Flowering takes place during February to April when the tree is more or less leafless whereas fruiting starts from May onwards up to June.

The fruit is up to 2.5 cm long, smooth, dark green, turning yellow when ripe and has a fruity smell.

DISTRIBUTION

In India, *Gmelina arborea* occurs extensively from the Ravi eastwards in the sub-Himalayan tracts, common throughout Assam and adjoining areas of northern West Bengal, also in southern Bihar and Odisha, sporadically found in western and southern India and planted elsewhere on a large scale. Gamhar most commonly occurs in West Bengal forests in mixed forests. It also occurs naturally in Myanmar, Thailand, Laos, Cambodia,Vietnam and in southern provinces of China.

MAJOR CHEMICAL CONSTITUENTS

Lignans, such as 6“ - bromo –isoarboreol, 4-hydroxysesamin, 4,8-dihydroxysesamin, 1,4-dihydroxysesamin (gummadiol), 2-piperonyl-3-hydroxymethyl-4-(α-hydroxy-3,4-methylenedioxybenzyl)-4-hydroxytetrahydrofuran and the 4-O-glucoside of 4-epigummadiol, can be isolated from the heartwood of *Gmelina arborea* (A S R Anjaneyulu *et al*.,1977). The parent compounds are arboreol or gmelanone(A S R Anjaneyulu *et al*., 1975).

Presence of Umbelliferone 7-apiosylglucosidecan on the root is reported (P Satyanarayana et al., 1985). Other constituents present in plant are β-sitosterol, ceryl alcohol, gmelinol; butyric acid & tartaric acids; apigenin, premnazole, arborone, isoarboreol, cutytyl ferulate, epieudesmin, gmelanore, gmelafuran, gummadiol, saponifiable fraction, apiosyl-skimmin, octacosanol etc.

TRADITIONAL USES

The bark of *Gmelina arborea* are stomachic, galactogogue, laxative and antihelmenthic, improve appetite, useful in hallucination, piles, abdominal pains, burning sensations, fever, 'Tridosha' and urinary discharge. It is also recommended with other drugs for the treatment of snake bite and scorpion sting.

- Leaf paste is applied to relieve headache and juice is used as wash for ulcers. Gamhar leaves, Apamarga roots and bark skin of Saimali are mashed with cow's milk and are given orally to treat hyperacidity. The leaves juice, milk and sugar are recommended in inflammatory condition of urinary bladder and dysuria.
- Flowers are sweet, cooling, bitter, acrid and astringent which is useful in leprosy and blood diseases.
- Fruit is acrid, sour, bitter, sweet, cooling, diueretic, tonic, aphrodisiac, promote growth of hairs, useful in 'Vata', thirst, anaemia, leprosy, ulcers and vaginal discharge. It is also recommended in raktapitta, excessive thirst, sexual debility in males and habitual abortion. The ripened fruit is valuable in heart disease of vata imbalance.
- The roots are described in the ayurvedic tests as mild laxatives which treats flatulence and increase appetite, lactation and reliever of menstrual irregularities. The cold infusion of candana, ustra and menstrual gamhar works well with sugar to alleviate the thirst. It is also usefull in piles, burning sensation, fever and 'Tridosha'.

IMPORTANT PREPARATIONS

Dasamularishta, Sriparni taila; Draksadi Kvatha, Kasmaryadi panaka, Kasmarya rasayana, Kasmarya taila.

PHARMACOLOGICAL STUDIES

1. In a study, the cytotoxicity of ethanolic leaf extracts of *Gmelina arborea* (Verbenaceae) was tested against Colon cancer (COLO 201), Gastric cancer (HT-29) and Human oesophagel cancer (TE-2) cell lines using the thiazolyl blue test (MTT) assay. Ethanolic leaf extracts of G. arborea was exhibited a prominent inhibitory effect against COLO 201 (IC 50- 20±0.15

mg/ml), HT-29 (IC 50-12±0.32 mg/ml) and TE-2 (IC 50- 16±0.05mg/ml) under in vitro condition. From the results it could be found that G. arborea ethanolic leaf extract has potent in vitro cytotoxic activity (David Punitha *et al* 2012).

2. In a study the anti-inflammatory and anti-nociceptive properties of aqueous extracts (AE) and methanol extracts (ME) of *G. arborea* was evaluated. Anti-inflammatory activity was determined in Wistar albino rats in acute inflammation model induced by carrageenan. And anti-nociceptive activity was evaluated by using hot plate test and writhing test in Swiss albino mice. The findings suggested that *G. arborea* possess significant anti-inflammatory and anti-nociceptive activities (Yogesh *et al* 2013).
3. The effect of *Gmelina arborea* bark and fruit aqueous extracts on paraquat- and hydrogen peroxide–induced oxidative stress was investigated using liver slice culture. Both paraquat and hydrogen peroxide were found to be cytotoxic as measured by release of lactate dehydrogenase from liver slice culture. Addition of bark and fruit extracts along with these cytotoxic agents led to a decrease in lactate dehydrogenase release. Addition of the plant extracts along with the pro-oxidants suppressed the enzyme activities. The extracts also displayed antioxidant activity in in vitro radical scavenging assays. Results indicate that Gmelina bark and fruit extracts protected liver slice culture cells by alleviating oxidative stress induced damage to liver cells (Sangeetha *et al* 2006).
4. In a study, the immunomodulatory effects of roots of *Gmelina arborea* Linn. were investigated. Methanolic extract of *G. arborea* Linn. (MEGA) and its ethyl acetate fraction (EAFME) were used and the modulating effect was evaluated on humoral and cell-mediated immune response using animal models like cyclophosphamide-induced myelosuppression, delayed-type hypersensitivity (DTH) response, and humoral antibody (HA) titre. Both test extracts produced significant increase in HA titre, DTH response, and levels of total white blood cell count and the drug was found to be a potential immunostimulant (Shukla *et al* 2010).
5. In another study the analgesic activity of different extracts of fruits of *Gmelina arborea* using ethanol, ethyl acetate and n-butanol as solvents were evaluated. The analgesic activity was determined by tail immersion and acetic acid induced writhing response method using Swiss albino mice as animal model. The extracts were found as non-toxic. The ethanol and petroleum ether extracts showed significant analgesic activity as compared to standard drugs

Pentazocine and Indomethacine. It could be concluded that G. arborea fruits possess analgesic activity (Bhabani *et al* 2014).

MATERIALS

I. COLLECTION OF PLANT MATERIAL

***Plant material*:-**

Binomial	: *Gmelina arborea*
Family	: Verbenaceae

Common name	: Gamhar
Habit	: Tree
Parts used	: Stem Bark

Collection:- The stem bark of *Gmelina arborea* (GA) was collected from and authentified by the Department of Botany. The plant material was dried at 45-50ºC for one week and powdered.

II. ANIMALS

Male Swiss Albino mice (6 to 8 weeks old) weighing 25-30g was used for the present study. The animals were housed in well ventilated polypropylene cages under controlled temperature (22-25^{0}C), relative humidity (60-80%) and light-dark cycle of 12 hrs. They were provided with mice feed and water *ad libitum.* All the animal experiments were carried out in RVS College of Pharmaceutical Sciences, according to the rules and regulations of Institutional Animal Ethics Committee (IAEC).

III. CELL LINES

Dalton's lymphoma ascites tumor cells (DLA) and Ehrlich ascites carcinoma cells (EAC) maintained in Amala Cancer Research Centre were used for the assay.

Maintaining of tumor cell lines:-

Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells were maintained in the ascites fluid of the peritoneal cavity of mouse. The aspirated tumor cells were washed 3 times with PBS (pH-7.4) and adjusted the cell count to 1×10^6 cells/ml using haemocytometer. Approximately, 1×10^6 cells were injected intraperitonially to develop ascites tumor in mice.

METHODS

1. PREPARATION OF EXTRACT

The powdered plant material was extracted with 70% methanol using a Soxhlet extraction system. The extract was filtered and evaporated to dryness. The colour, and the % yield of the extract were noted. The dried extract was re-dissolved in distilled water and used for further studies.

2. EVALUATION OF ANTI-INFLAMMATORY ACTIVITIES

2.1. DEXTRAN INDUCED ACUTE INFLAMMATION

Male Swiss Albino mice (25-30) were divided into 4 groups of 7 animals each.

Group 1 – Control – 1% dextran alone

Group 2 - Standard -10mg/Kg Diclofenac + 1% Dextran

Group 3 - GALC - GA low dose (250mg/Kg body. wt) + 1% Dextran

Group 4 - GAHC - GA high dose (500mg/Kg body .wt) + 1% Dextran

The animals were pretreated with respective drug doses for 3 days. On the 4th day exactly 1hr after administration of GA extract or Diclofenac, all the animals were injected subplantarly into right hind paw with 0.02 ml of 1% suspension of Dextran in 0.1% carboxy methyl cellulose to induce inflammation. Paw volume was measured 1hr prior and for 5hr after Dextran administration using vernier caliper. The percentage of inhibition was calculated according to the following formula

% inhibition = [(VT –V0)Control - (VT –V0)] treated group (VT –V0)* 100

Where, VT = Paw oedema at various time intervals; V0 = initial paw oedema

2.2. FORMALIN INDUCED CHRONIC INFLAMMATION

Male Swiss Albino mice (25-30) were divided into 4 groups of 7 animals each.

Group 1 – Control – 1% Formalin alone

Group 2 - Standard -10mg/Kg Diclofenac + 1% Formalin

Group 3- GALC - GA low dose (250mg/Kg body. wt)+ 1% Formalin

Group 4- GAHC - GA high dose (500mg/Kg body .wt)+ 1% Formalin

All the animals were injected subplantarly into right hind paw with 0.02 ml of 1% suspension of Formalin. The animals were treated with respective doses of GA extract / standard drug Diclofenac one hour prior to formalin injection and continued for next 6 days. The paw volume was measured 1hr prior and for 6 days after formalin administration.

% inhibition = [(VT –V0)Control - (VT –V0)] treated group (VT –V0)* 100

Where, VT = Paw oedema at various time intervals; V0 = initial paw oedema

3. *IN VITRO* CYTOTOXICITY ANALYSIS:-

Determination of short term *in vitro* cytotoxicity of *Gmelina arborea* stem bark extract by trypan blue dye exclusion method.

Principle:-

Viable cells exclude the dye while non-viable cells take up the dye and will appear in blue color under magnification by a microscope.

Materials required:-

Dalton's lymphoma ascites cells and Ehrlich ascites carcinoma cells maintained in Amala Cancer Research Centre, phosphate buffer saline (PBS pH 7.4), trypan blue and Hemocytometer.

Preparation of Phosphate Buffer Saline (PBS):-

NaCl	-	4g
KCl	-	0.1g
$Na_2HPO_4.2H_2O$	-	0.72g
KH_2PO_4	-	0.1g

Distilled water	-	500ml
pH	-	7.4

Procedure:-

Short-term cytotoxicity activity of the *G.arborea* methanol extract was assayed by determining the percentage viability of DLA and EAC cells using the trypan blue dye exclusion technique (Moldeus *et al*, 1978). DLA and EAC cells were cultured in the peritoneal cavity of healthy albino mice weighing between 25 to 30g by injecting a suspension of cells (1×10^6 cells/ml), intraperitonially. Tumor cells were aspirated aseptically from the peritoneal cavity of tumor bearing mice on day 15, washed with PBS for 3 times and cells were suspended in 1ml PBS (0.1ml cells +0.9ml) and adjusted the cell number to 1×10^6 cells/ml. The viability of the cells was checked using trypan blue stain (0.1ml cells+ 0.9ml PBS+ 0.1ml trypan blue) with Hemocytometer. The cells were incubated at 37^0C for 3 hours with different concentration of drug with 1×10^6 tumor cells. After incubation, 0.1ml trypan blue were added to determine the number of dead cells using Hemocytometer and substituting in the equation:

$$\text{Percentage of identity of cytotoxicity} = \frac{\text{Number of dead cells}}{\text{Number of total cells}} \times 100$$

4. *IN VIVO* ANTI-TUMOR ANALYSIS

For assessing the antitumor activity, Dalton's Lymphoma Ascites (DLA) cell induced solid tumor model and Ehrlich Ascites Carcinoma (EAC) cell induced ascites tumor model were employed.

4.1. EAC INDUCED ASCITES TUMOR MODEL SYSTEM:-

Male Swiss Albino mice were grouped into four groups (6 animals per each group).

Group I: Control – Untreated;

Group II: Standard – Cyclophosphamide 10mg/kg;

Group IV: GALC – GA extract low concentration (250 mg/kg b.wt);

Group V: GAHC - GA extract high concentration (500 mg/kg b.wt).

EAC cells were aspirated from peritoneal cavity of the tumor bearing mice and 0.1ml containing $1x10^6$ cells/ml was injected intraperitoneally into all the animals. The drug administration has started next day after the induction of tumor and continued for 10 consecutive days. The animals were observed for the development of ascites tumor and death due to tumor burden was recorded for 30 consecutive days. The life span of animals was calculated using the formula,

%ILS = (T-C)/C × 100, where T and C are mean survival of treated and control mice respectively (Mazumdar UK, 1997).

4.2. DLA INDUCED SOLID TUMOUR MODEL SYSTEM:

4.2.1. Assessment of tumor volume:

Male Swiss Albino mice were grouped into four groups (6 animals per each group).

Group I: Control – Untreated;
Group II: Standard – Cyclophosphamide 10mg/kg;
Group IV: GALC – GA extract low concentration (250 mg/kg b.wt);
Group V: GAHC - GA extract high concentration (500 mg/kg b.wt).

DLA cells were aspirated from peritoneal cavity of the tumor bearing mice and 0.1ml containing $1x10^6$ cells/ml was injected intramuscularly into the right hind limb of all the animals. The drug administration was started next day after the induction of tumor and continued for 10 consecutive days. The tumor development on animal in each group was determined by measuring the diameter of the tumor growth in two perpendicular planes using a vernier caliper at fixed intervals (on each 3rd day) and the volume was calculated using the formula,

Tumor volume = $4/3\ \pi\ r_1^2 \times r_2$ where r_1 is the minor radius and r_2 is the major radius.

And the percentage of inhibition of tumor volume in animals was calculated by,

% of inhibition =[(tumor volume of control on 30th day – Tumor volume of treated on30th)/(Tumor volume of control on 30th day)] × 100

4.2.2. Comparison of haematological parameters between cyclophosphamide treated and *G. arborea* treated animals

As part of the DLA induced solid tumor model study, the haematological parameters of the animals were tracked on every 3rd day. For this, blood was collected from the caudal vein into heparinised tubes and total WBC count and haemoglobin level were checked.

4.2.2.1. *Determination of total WBC count:-*

Principle:

The whole blood was diluted using a diluent which haemolyses red cells, leaving all the nucleated cells intact. The number of white cells in a known volume and known dilution were counted using a counting chamber.

Preparation of Diluting Fluid

Glacial acetic acid	- 2ml
1% crystal violet (1g in 100ml)	- 1ml
Distilled water	- 97ml

Stir overnight, filter and store.

Procedure:

0.02 ml of blood was added to 0.38 ml of diluting fluid and mixed well. The diluted blood was charged into a Neubauer counting chamber. After 3-4 min, the total number of white blood cells in the four large corner square chambers was counted.

Calculation:

Total WBC = (Number of cells counted x 50) / mm^3

4.2.2.2. Determination of haemoglobin (Hb) content:- Cyanmethaemoglobin method

Principle:-

Haemoglobin was treated with a reagent containing potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate. The ferricyanide forms methaemoglobin, which is converted to cyanmethaemoglobin by cyanide. The intensity of colour formed is measured at 546 nm against blank. The optical density is directly proportional to the amount of haemoglobin present in blood.

Requirements:- Kit manufactured from Agappe Diagnostics

Procedure:-

0.02 ml of fresh whole blood was mixed with 5 ml of the cyanmeth reagent. The optical density was measured at 546 nm against blank after 5 min incubation at room temperature. The OD of standard solution corresponding to 60 mg/dl haemoglobin at 546 nm was also read against reagent blank.

Calculation:-

Haemoglobin (g/dL) = (OD of treated x 60 x 0.251) / OD of standard

STATISTICAL ANALYSIS

The values were expressed as mean±SD. Statistical evaluation of the data was done by one way ANOVA followed by Dunnett't' test using graph pad instat 3 software. Results were considered statistically significant when $p<0.05$.

RESULTS

1. Extraction of *Gmelina arborea* stem bark

The stem bark of *G. arborea* were extracted using 70% methanol and the yield was 9.5% W/W (Tab.5).

Tab. 5 : Characteristics of *Gmelina arborea* stem bark extract

Name of Extract	Colour	Consistency	Yield (% W/W)
70% Methanolic Extract	Black	Sticky	9.5

2. *In vivo* Anti inflammatory analysis

2.1. Dextran induced acute inflammation

The *Gmelina arborea* high dose (GAHC) group significantly reduced the paw thickness by 34.14% in the 3rd hour when compared to the control group. GALC dose group reduced the paw edema by 15.36%. The percentage inhibition in standard (Diclofenac, 10mg/Kg) group was 21.34% (Tab.6 & Fig. 3).

Tab.6: *In vivo* anti-inflammatory effect of *G. arborea* stem bark extract on Dextran induced acute inflammation.

Groups	Initial Paw thickness (cm)	Paw thickness on 3rd hour (cm)	Increase in paw thickness (cm)	% of inhibition
Control	0.253±0.019	0.417±0.0091	0.164	
Standard	0.253±0.015	0.3826±0.0162[c]	0.129267	21.34146
GALC	0.2445±0.0152	0.383±0.0178[c]	0.138833	15.36585
GAHC	0.24725±0.023	0.3558±0.019[c]	0.108583	34.14634

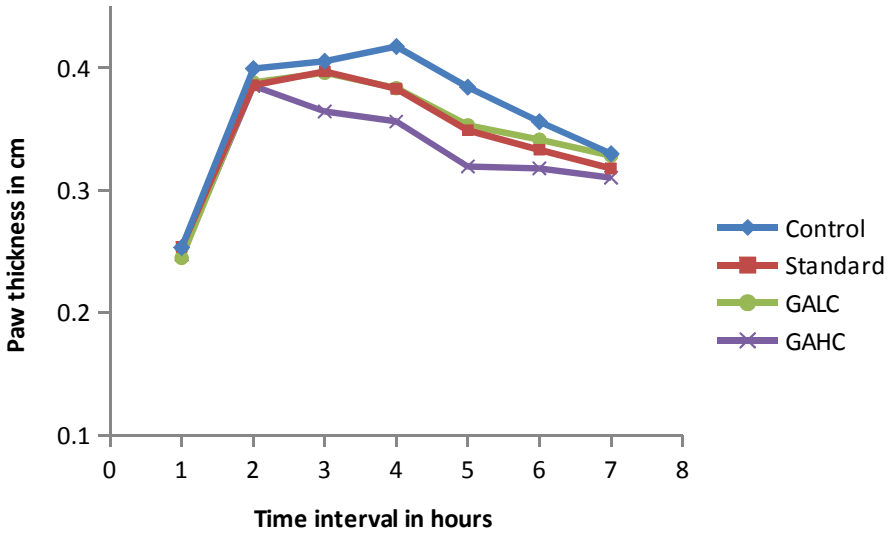


Fig.3: *In vivo* anti-inflammatory effect of *G. arborea* stem bark extract on Dextran induced acute inflammation. Values are expressed as mean ± SD for 6 animals

2.2. Formalin induced Chronic inflammation

The percentage inhibition of paw edema was 18.5% for *Gmelina arborea* low dose treated group and 34.07% in *Gmelina arborea* high dose treated group. The percentage inhibition in standard group was 45.92% (Tab. 7 & Fig. 4).

Tab.7 : *In vivo* anti-inflammatory effect of *G. arborea* stem bark extract on Formalin induced chronic inflammation.

Groups	Initial Paw thickness (cm)	Paw thickness on 6th day (cm)	Increase in paw thickness (cm)	% of inhibition
Control	0.268±0.0098	0.403±0.0139	0.135	
Standard	0.2644±0.0115	0.3383±0.0108^{c}	0.073933	45.92
GALC	0.2656±0.013	0.376±0.0146^{c}	0.110333	18.51
GAHC	0.2723±0.0106	0.3615±0.0119^{c}	0.089167	34.07

Values are expressed as mean ± SD for 6 animals: c: - p<0.01

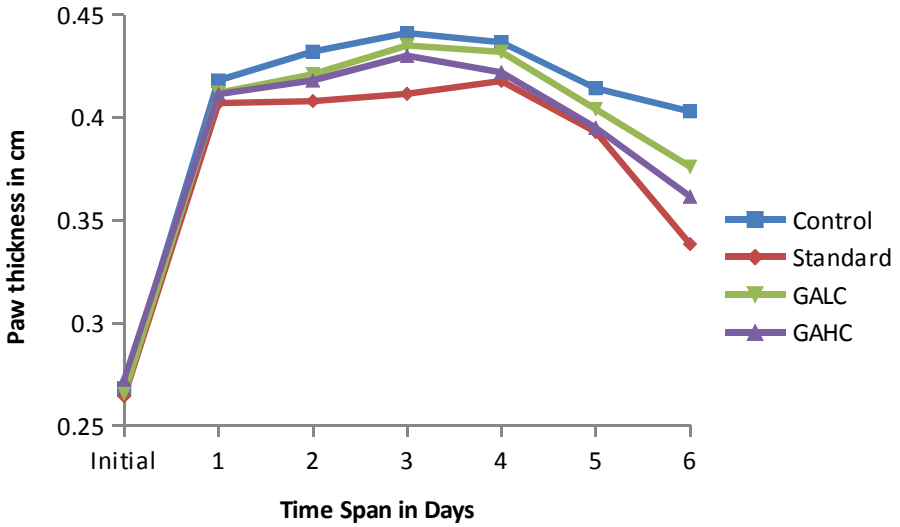
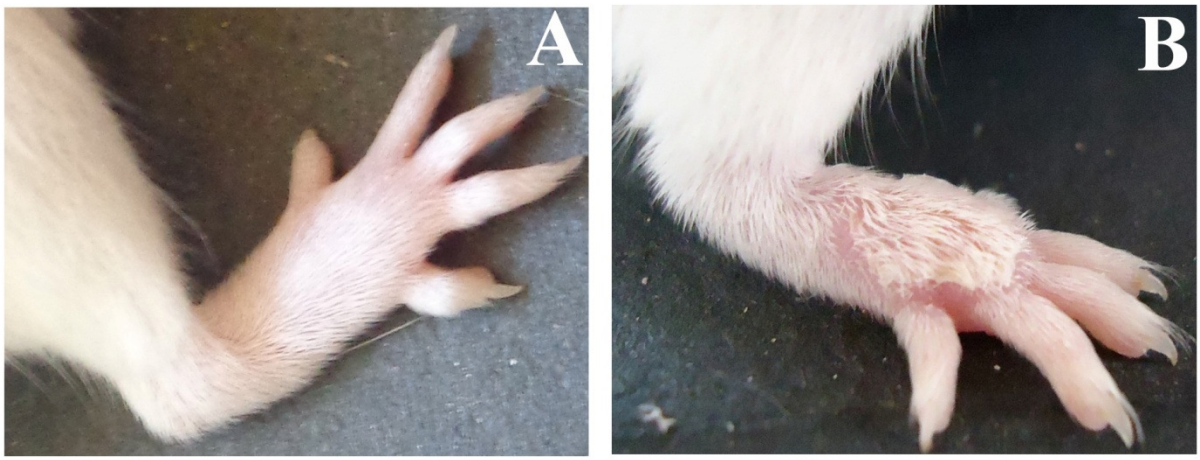


Fig. 4: *In vivo* anti-inflammatory effect of *G. arborea* stem bark extract on Formalin induced chronic inflammation. Values are expressed as mean ± SD for 6 animals

Fig. 5: Animal models for Dextran induced acute inflammation and Formalin induced chronic inflammation.



A: - Dextran induced acute inflammation; B: - Formalin induced chronic inflammation

3. *In vitro* Cytotoxic Analysis

3.1. Cytotoxic effect of *Gmelina arborea* on DLA cell lines

The methanol extract of *Gmelina arborea* stem bark showed marked cytotoxic activity against DLA cell lines, in a dose dependent manner (Fig. 6). The concentration required for 50% death (IC_{50}) was found to be 729.02µg/ml for DLA cells.

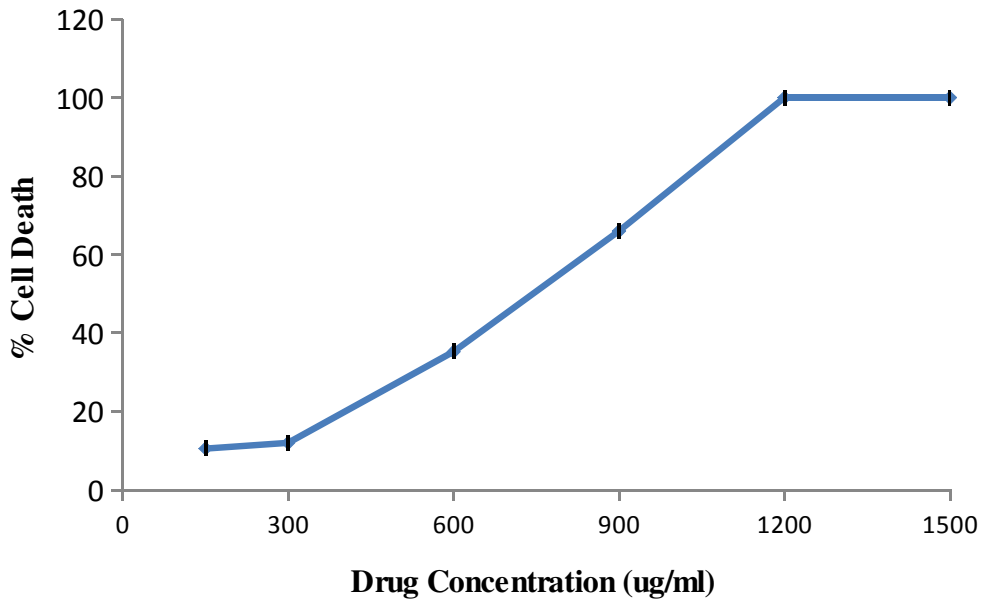


Fig. 6: *In vitro* cytotoxic effect of *G. arborea* stem bark extract on DLA cell lines. Values are expressed as mean ± SD for 4 experiments.

3.2. Cytotoxic effect of *Gmelina arborea* on EAC cell lines

The methanol extract of *Gmelina arborea* caused considerable mortality of EAC cells in the *in vitro* cytotoxicity assay, following the trypan blue exclusion method (Fig. 7). Incubation with increased concentrations of the extract produced a relational increase in cell death. The IC_{50} value was determined to be 849.49 µg/ml for EAC cells.

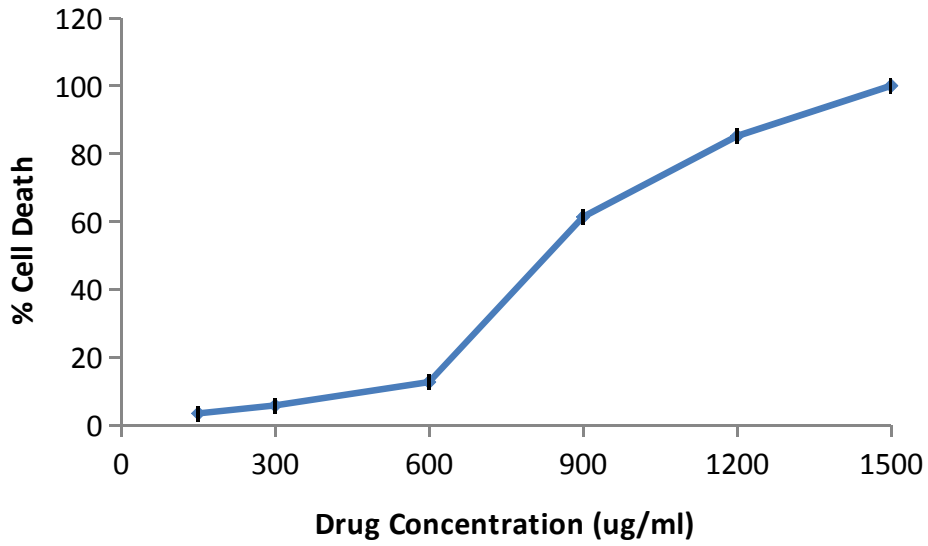


Fig. 7: *In vitro* cytotoxic effect of *G.arborea* stem bark extract on EAC cell lines. Values are expressed as mean ± SD for 4 experiments.

4. *In vivo* Antitumor analysis

4.1. Effect of *Gmelina arborea* extract on average life span in ascites tumor induced models.

The animals of the tumor control group inoculated with EAC cells survived for a period of 19.28± 1.79 days. The animals treated with cyclophosphamide survived for 27.42± 0.97days. The *Gmelina arborea* methanolic extract at 250 and 500 mg/kg body weight increased the average life span of animals by 22.14± 1.06 days and 24.5± 1.64 days respectively. Both were significantly high ($p<0.01$), in comparison with the control. The *Gmelina arborea* methanolic extract at 500 mg/kg body weight was found to be more inhibiting the proliferation of EAC cells with the percentage increase in life span by 27.04 % than the GALC extract (14.81 %). However, in cyclophosphamide treated mice (10 mg/kg), the percentage increase in life span was found to be the highest (42.22 %) (Tab. 8 & Fig. 8).

Tab.8: Effect of *Gmelina arborea* stem bark treatment on average life span of ascites tumour bearing mice.

Groups	Mean survival days	% ILS
Control	19.28±1.79	
Standard	27.42±0.97[c]	42.22
GALC	22.14±1.06[c]	14.81
GAHC	24.5±1.64[c]	27.04

Values are expressed as mean ± SD for 6 animals: c: - p<0.01, when compared to control.

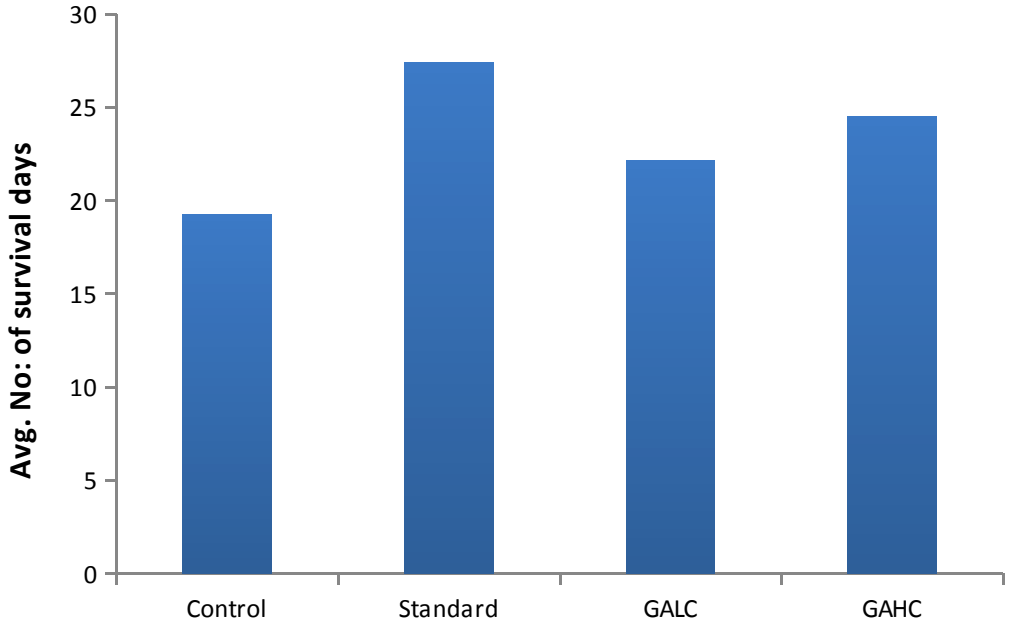


Fig.8: Effect of *Gmelina arborea* stem bark treatment on average life span of ascites tumour bearing mice. Values are expressed as mean ± SD for 6 animals; c:- p<0.01 when compared to control.

4.2. Effect of *Gmelina arborea* methanolic extract on tumor volume in solid tumor induced models.

4.2.1. Assessment of tumour volume

The animals injected with DLA cell lines alone showed marked increase in tumor volume on the 30th day of inoculation in control group. The tumor volume in the mice treated with GA extract at dosages of 500 mg/kg b.wt and 250 mg/kg b.wt. on the 30th day of inoculation decreased significantly by 49.78 % and 65.54 % respectively. The tumor volume had decreased by 74.83 % in cyclophosphamide (10mg/kg) treated group (Tab. 9 & Fig. 9).

Tab.9: Effect of *Gmelina arborea* stem bark treatment on average solid tumour volume

Groups	Tumor volume on 30 th day (cm^3)	% Inhibition
Control	1.639±0.125	
Standard	0.412±.006[c]	74.83151
GALC	0.823±0.085[c]	49.78437
GAHC	0.564±0.025[c]	65.54948

Values are expressed as mean ± SD for 6 animals: c: - p<0.01 when compared to control.

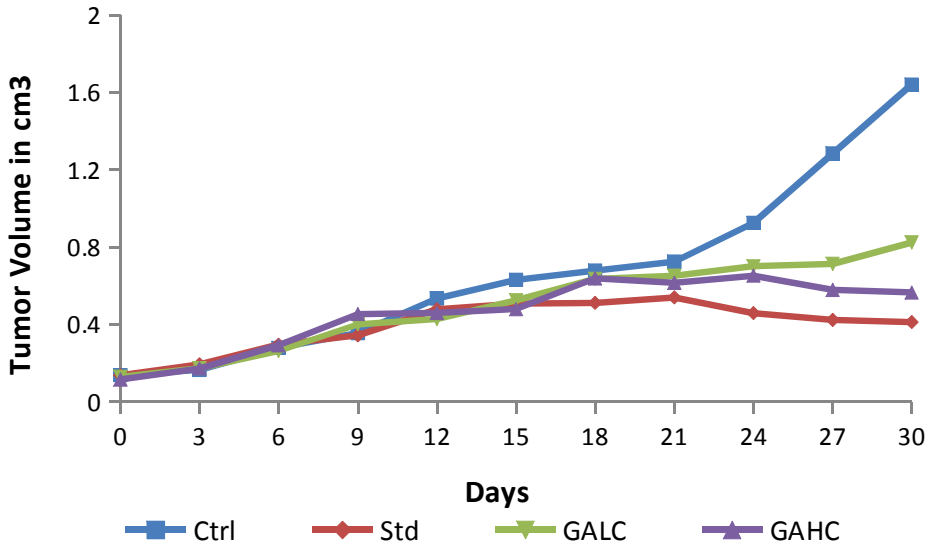


Fig. 9: Effect of *Gmelina arborea* stem bark treatment on average solid tumour volume.

Values are expressed as mean ± SD for 6 animals:

4.2.2. Comparison of haematological parameters in cyclophosphamide and *Gmelina arborea* treated tumor models.

4.2.2.1. Total WBC count:-

Fig. 10 & Tab. 10 shows that cyclophosphamide treatment led to a decline in the total WBC count over the period of study, which is also believed to be one of the contra indications of its use. But such reduction of leukocyte count was not observed in *Gmelina arborea* treated animals, as evident from the count taken on the 30th day. Cyclophosphamide administration brought down the values to 12400 ± 557.73cells/mm^3, towards the end of the study, which was significantly very low ($p<0.01$), as compared to the control $p<0.05$, GALC $p>0.05$ and GAHC $p>0.05$ groups.

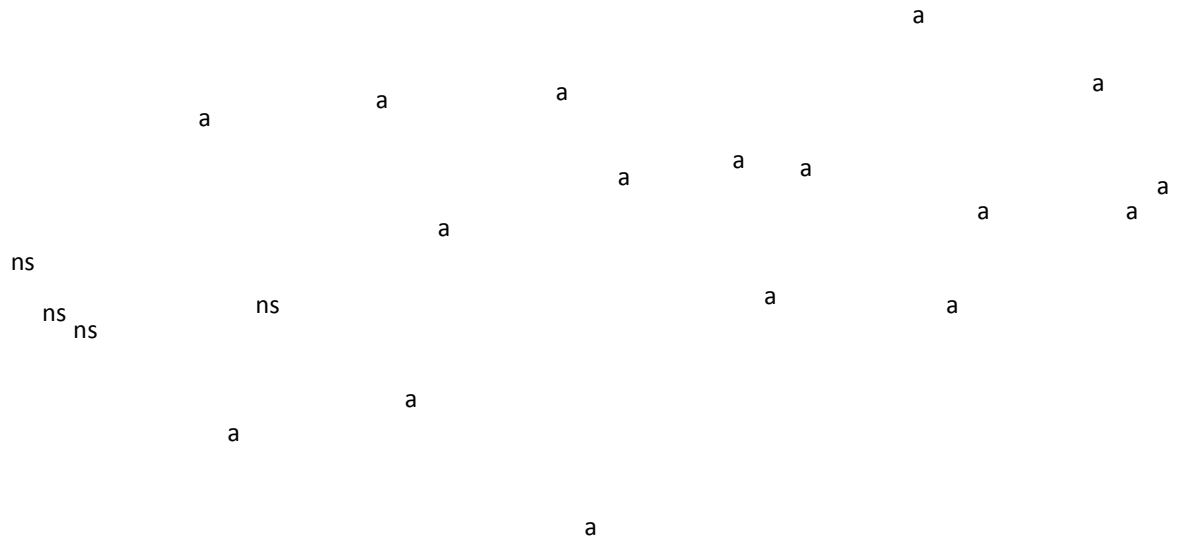


Fig. 10: Effect of *Gmelina arborea* stem bark treatment on total WBC count. Values are expressed as mean ± SD for 6 animals: a: - $p<0.01$ and ns: - non significant, when compared to standard.

Tab.10: Effect of *Gmelina arborea* stem bark treatment on total WBC count.

Days						
0	**5**	**10**	**15**	**20**	**25**	
20534.3±453.4[ns]	21416.67±708.59[a]	21233.33±605.50[a]	22143±552.75[a]	21600±1052.75[a]	22716.67±673.20[a]	22166
19965.4±567.5	19900±401.3	16966.67±731.5	15885±541.66	14416.67±777.2	13350±824.57	124
20105.2±342.7[ns]	14480±945.12[a]	17200±635.16[a]	17460.45±554.57[a]	18300±841.75[a]	22400±564.57[a]	215
19938.7±334.2[ns]	20200±334.14[ns]	20616.67±434[a]	21045.62±632.75[a]	21466.67±422.7[a]	20283.33±651.66[a]	213

Values are expressed as mean ± SD for 6 animals: a: - $p<0.01$ and ns: - non significant, when compared to standard

4.2.2.2. Haemoglobin content:-

Though it was evident that animals treated with cyclophosphamide (10 mg/kg b.wt) exhibited substantial anti- tumour activity in solid tumour induced mice models, there was marked reduction in the haemoglobin content of their blood estimated on the 30th day (9.86 ± 0.259 g/dl) (Fig.11 & Tab. 11). This was significantly different from the control (12.23 ± 0.255 g/dl), GALC (13.21 ± 0.489 g/dl) and GAHC groups (13.80 ± 0.194 g/dl) ($p<0.01$). As seen from Fig. 11, the cyclophosphamide treated group alone showed a sharp decrease in haemoglobin levels over a time period of 30 days. Treatment with *G. arborea* stem bark extract didn't show such reduction in Hb, besides being mediation in reducing solid tumour volume.

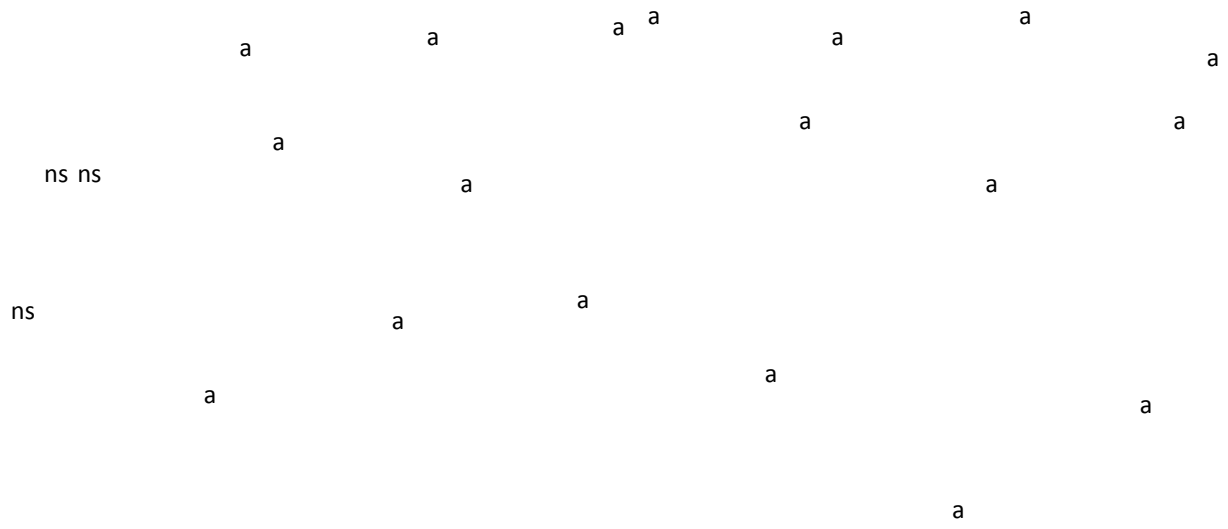


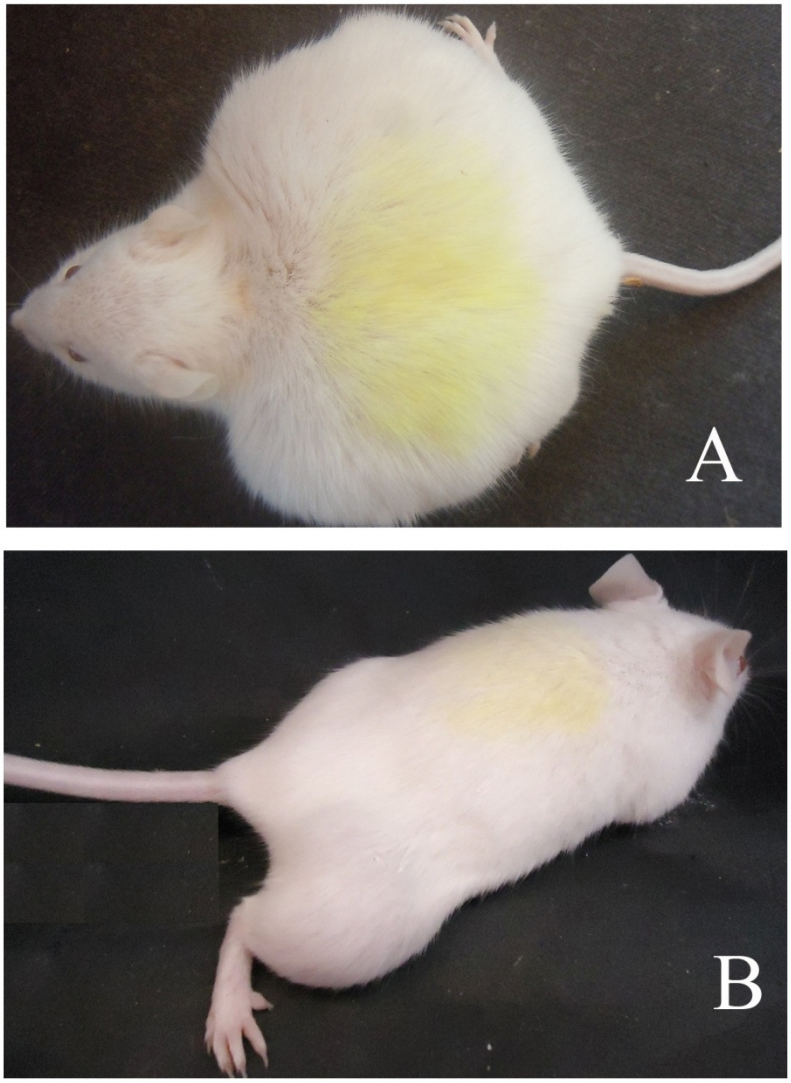
Fig.11: Effect of *Gmelina arborea* stem bark treatment on haemoglobin levels. Values are expressed as mean ± SD for 6 animals: a: - $p<0.01$ and ns: - non significant, when compared to standard.

Tab.11: Effect of *Gmelina arborea* stem bark treatment on haemoglobin levels.

Treated Groups	Days						
	0	**5**	**10**	**15**	**20**	**25**	**30**
Control	12.67±0.132^{ns}	12.408±0.112^{a}	12.565±0.29^{a}	12.63±0.313^{a}	12.398±0.235^{a}	11.930±0.098^{a}	12.23±0.255^{a}
Standard	13.04±0.238^{ns}	10.554±0.112^{a}	10.254±0.426^{a}	10.543±0.328^{a}	10.164±0.641^{a}	10.069±0.432^{a}	9.86±0.259^{a}
GALC	13.26±0.248^{ns}	13.61±0.435^{a}	13.573±0.528^{a}	13.489±0.654^{a}	13.420±0.278^{a}	13.226±0.118^{a}	13.217±0.489^{a}
GAHC	13.10±0.385^{ns}	13.034±0.598^{a}	12.946±0.485^{a}	13.453±0.739^{a}	13.879±0.213^{a}	13.770±0.411^{a}	13.804±0.194^{a}

Values are expressed as mean ± SD for 6 animals: a: - $p<0.01$ and ns: - non significant, when compared to standard

Fig. 12: Animal models for DLA induced solid tumor and EAC induced ascites tumor.



A: - EAC induced ascites tumor model, B:- DLA induced solid tumor model.

DISCUSSION

Medicinal plants and their phytochemicals, are reported to possess substantial antioxidant, anti-inflammatory anticarcinogenic activities. These phytochemicals have been explored extensively for their potential in the treatment of cancer. Well known compounds like paclitaxel, a diterpinoid from *Taxus brevifolia*, and vincristine, an alkaloid from *Catharanthus roseus* (Wall *et*

al., 1996), semisynthetic derivatives (Topotecan and Irinotecan) of alkaloid camptotecan from *Camptotheca accuminata* (Rahier *et al*., 2005) are used in chemotherapy today.

In this study methanolic extract of *Gmelina arborea* is evaluated for its anti cancer and anti inflammatory activity. *Gmelina arborea* Roxb (Verbenaceae) is a well-known medicinal plant in Ayurveda, an ancient Indian system of medicine. The roots, leaves, flowers, fruits and bark are used for treating different disorders in traditional medicine. There are reports on phytoconstituents present in different parts of the plant. The isolation of luteolin (Rao *et al*., 1969) and indole alkaloids (Bhattacharjee *et al*., 1969) from the leaves has been reported. Hentriacontanol (Joshi *et* al., 1971) and lignans such as arboreol, isoarboreol, methyl arboreol, arborone, gmelanone, gummadiol, and 7-oxodihydrogmelinol (Govindachari *et al*., 1972) have been isolated from heartwood of the plant. Few coumarin glycosides (Satyanarayana *et* al., 1985) and iridoid glycosides (Hosny *et* al., 1998) have been reported in roots and leaves, respectively. Three iridoid glycosides 6-*O*-(3"-*O*-benzoyl)-**α**-L-rhamnopyranosylcatalpol, 6-*O*-(3"-*O*-trans-cinnamoyl)-**α**-L-rhamnopyranosylcatalpol and 6-*O*-(3"-*O*-cis-cinnamoyl)-**α** L rhamnopyranosylcatalpol were isolated from aerial parts of *G. arborea* (Tiwari *et al*., 2008). A study reported the presence of apigenin in the bark (Vidya *et al*., 2011), tyrosol [2-(4-hydroxyphenyl) ethanol]; (+)-balanophonin, an 8-5′ neolignan, gmelinol, phenylethanoid glycoside {(−)-*p*-hydroxyphenylethyl [5"′-*O*-(3,4 dimethoxycinnamoyl)-b-D-apiofuranosyl (1"′ → 6')]-b-*D*-glucopyranoside}, 2,6-dimethoxy-*p*-benzoquinone and 3, 4, 5-trimethoxyphenol (Syamsul *et al*., 2008).

On the basis of the results obtained from the study, it is concluded that a methanolic extract of *Gmelina arborea* stem bark, which contains large amounts of phenolic compounds, exhibits high antioxidant activities. The *in vitro* assays indicates that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses (Patil *et al*., 2009). Since the plant possesses antioxidant activity it may enhance the anticancer effects without causing side effects when compared to chemotherapeutic agents.

Inflammation is a part of the complex biological responses of vascular tissues to harmful stimuli such as irritants, damaged cells, or pathogens (Ferrero-Miliani *et al*., 2007). Inflammation can be classified as acute or chronic. Acute inflammation is initial response of the body to

harmful stimuli and is brought by the increased movement of leukocytes and plasma from the blood into the injured tissues. A series of biochemical events are propagated and the inflammatory response matures, involving the immune system, local vascular system and various cells within the injured tissue. The common signs of acute inflammation are pain, swelling, redness, heat, and loss of function. Prolonged inflammation which is known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

The association between inflammation and cancer was illustrated by clinical studies (Balkwill *et al*., 2001 and Coussens *et al*., 2002). For instance, the risk of colorectal cancer was 10-fold greater if linked with inflammatory bowel disease, such as Crohn's disease and ulcerative colitis (Tzkowitz *et al*., 2004). Moreover, the control of colitis by certain anti-inflammatory agent reduced colon cancer incidence (Moody *et al*., 1996). It was also suggested that cancer risk is positively associated with the severity and duration of inflammatory diseases (Keeley *et al*., 1997). The cause of inflammation may be microbial infection or a non infective physical or chemical irritant (Philip *et al*., 2004). Chronic inflammation not caused by infection may also lead to carcinogenesis and the risk of esophageal cancer, pancreatic cancer, and gall bladder cancer may be increased by inflammatory diseases such as chronic pancreatitis, Barrett's metaplasia and esophagitis, (Macarthur *et al*., 2004 and Whitcomb, 2004). Possible associations were also found in Marjolin's ulcer and skin carcinoma, asbestos and mesothelioma , silica, cigarette smoke, and bronchial cancer (Macarthur *et al*., 2004), chronic asthma and lung cancer (Vesterinen *et al*., 1993 and Wu *et al*., 1995), sarcoidosis and lung, skin, and liver cancer (Askling *et al.,* 1999), ulcerative lichen planus and verrucous carcinoma (Carlson *et al*., 1998 and Mayrom *et al.,* 1998), foreskin inflammation and penile cancer (Perky., 1977), and pelvic inflammatory disease or ovarian epithelial inflammation and ovarian cancer (Risch *et al*., 1995). Chronic prostatitis, resulting from either persistent bacteria infection or noninfective stimuli, was associated with prostate cancer (Palapattu *et al*., 2004). Therefore, there is increasing evidence that supports the association between chronic inflammation and cancer development.

The present study establishes the anti inflammatory and anti tumour activity of *Gmelina arborea* stem bark extract in animal models. Formalin and Dextran induced paw edema as an *in vivo*

model of inflammation was frequently used to assess the effect of anti inflammatory activity of natural products. The clinical treatment of inflammatory diseases is dependent on non-steroidal or steroidal chemical therapeutics (Rainsford, 2007). However, long term administration of NSAID may induce gastrio-intestinal ulcers, bleeding and renal disorders (Robert, 1976 and Tapiero *et al*., 2002) and also the use of steroidal anti-inflammatory agents causes multiple side effects (Schacke *et al*., 2002 and Reinke *et al*., 2002). Therefore, developing new agents with more powerful anti-inflammatory activities with lesser side effects will be of great interest.

Dextran induced paw edema is a consequence of liberation of histamine and serotonin from mast cells while formalin induced paw edema is mediated by an early release of bradykinin followed by release of histamine, 5-hydroxytryptamine (5-HT) and prostaglandins (Wheeler *et* al., 1991). The involvement of bradykinin and some other inflammatory mediators in formalin-induced edema and plasma extravasation was examined. Various mediators are released in the paw and the release of the substances like cytokinin, histamine and serotonin results in enhanced vascular permeability and thereby promotes the accumulation of fluid in the tissues that accounts for the edema. In the present study, the dextran model showed reduction in paw edema ($P< 0.01$) with the administration of extract at doses of 250 and 500 mg/Kg b. wt. The results suggest that the extract may have an inhibitory effect on these mediators.

Formalin induced paw edema is one of the most suitable test procedure to screen anti-inflammatory agents. In *G. arborea* extract treated groups of animals a decrease in paw edema ($P< 0.01$) is found which revealed the protective activity of the extract in chroninc inflammatory condition. Anti inflammatory activity of various plant extracts has also been reported (Perez *et al*., 1995).

The anti cancer activity of *G. arborea* extract was also studied by *in vitro* cytotoxicity and *in vivo* animal models using two mouse cancer cell lines DLA and EAC. Since cancer is one of the major cause of death worldwide and the failure of conventional chemotherapy to effect a major reduction in mortality indicates that new approaches are critically needed (Balasubramanian *et al*., 2007). Plants possess various biological activities including anti inflammatory, anti oxidant and anti cancer activities (Lee *et al*., 2004). Some studies have reported that extracts from natural products such as fruits, vegetables and medicinal herbs have positive effects against cancer when

compared with chemotherapy or recent hormonal treatments (Pezzuto., 1997). Therefore many plants have been examined to identify new and effective antioxidant and anti cancer compounds as well as to elucidate the mechanisms of cancer prevention (Kim *et al*., 1998).

The results obtained from the cytotoxicity assay by trypan blue dye exclusion method indicates that the methanol extract of *Gmelina arborea* could inhibit the growth of DLA and EAC cells via exhibiting cytotoxic effect. Cytotoxicity is one of the chemotherapeutic targets of antitumor activity (Suffness *et al*., 1991). The cytotoxic activity of *Gmelina arborea* against DLA and EAC cell lines partially explains its significant anti tumor activity against solid and ascites tumor. The antitumor activity was evaluated in solid tumor model. Methanolic extract of *Gmelina arborea* reduced the tumor burden effectively.

The oral administration of the *G.arborea* extract at 250 and 500 mg/kg body weight for 30 days in mice considerably reduce DLA induced solid tumour volume by 49.78 % and 65.54 % respectively. The total WBC count and hemoglobin level in the *G.arborea* treated animals are similar to that of normal untreated mice. However, the standard drug, Cyclophosphamide shows significant reduction in total WBC count, and haemoglobin level. This result is very encouraging, as today majority of the tumour reducing drugs specifically show drug induced myelosuppression and other complications. Even though the standard drug, cyclophosphamide, used shows marked decrease in the tumor burden, it forms several side effects which may lead to the cause of death. In the present study, extract of *G. arborea* stem bark does not produce such lethal effects. In this state, we use phytochemicals as a remedy for surviving cancer without or with fewer side effects. They may not be as effective as standard drug, but are less toxic to normal cells and do not cause side effects. This seems to be advantageous over other chemotherapeutic drugs which impart side effects.

CONCLUSION

In this study the methanolic extract of the plant *Gmelina arborea* stem bark was evaluated for its anti inflammatory and anti tumour property. The anti inflammatory study was done in dextran and formalin induced inflammatory models, whereas the anti tumour activity was screened in solid and ascites tumour model in mice. The results clearly suggest that methanolic extract of *Gmelina arborea* possess significant anti inflammatory and anti cancer activity. The extract also showed *in vitro* cytotoxic effect on DLA and EAC cell lines. Further studies are required to

confirm the exact mechanism underlining anti inflammatory and anti cancer property of the extract and to identify the chemical constituents responsible for it.

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